Chromo4™ Detector

For Real-Time Fluorescence Detection

Operations Manual

Supports Opticon Monitor™ software version 2.03

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Explanation of Symbols

CAUTION: Risk of Danger! Wherever this symbol appears, always consult note in this manual for further information before proceeding. This symbol identifies components that pose a risk of personal injury or damage to the instrument if improperly handled.

CAUTION: Risk of Electrical Shock! This symbol identifies components that pose a risk of electrical shock if improperly handled.

CAUTION: Hot Surface! This symbol identifies components that pose a risk of personal injury due to excessive heat if improperly handled.

Safety Warnings

Warning: Operating the Chromo4 detector before reading this manual can constitute a personal injury hazard. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate this instrument.

Warning: Do not open or attempt to repair the Chromo4 unit. Doing so will void your warranties and can put you at risk for electrical shock. Return the Chromo4 detector to the factory (North American customers) or an authorized distributor (all other customers) if repairs are needed.

Warning: The sample block can become hot enough during the course of normal operation to cause burns or cause liquids to boil explosively. Wear safety goggles or other eye protection at all times during operation.

Caution: Never remove the Chromo4 unit from the DNA Engine base when the power is turned on or a program is running. Doing so can cause electrical arcing that can melt the contacts in the connector joining the Chromo4 unit to the cycler.

Safe Use Guidelines

The Chromo4 system is designed to operate safely under the following conditions:

- Indoor use
- Altitude up to 3000m
- Ambient temperature 15–31°C
- Humidity 10–80%, noncondensing
- Transient overvoltage per Installation Category II, IEC 664
- Pollution degree 2, in accordance with IEC 664

Electromagnetic Interference

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

This device has been tested and found to comply with the EMC standards for emissions and susceptibility established by the European Union at time of manufacture.

This digital apparatus does not exceed the Class A limits for radio noise emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

LE PRESENT APPAREIL NUMERIQUE N'EMET PAS DE BRUITS RADIOELECTRIQUES DEPASSANT LES LIMITES APPLICABLES AUX APPAREILS NUMERIQUES DE CLASS A PRESCRITES DANS LE REGLEMENT SUR LE BROUILLAGE RADIOELECTRIQUE EDICTE PAR LE MINISTERE DES COMMUNICATIONS DU CANADA.

FCC Warning

Warning: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio-frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Note Regarding FCC Compliance: Although this design of instrument has been tested and found to comply with Part 15, Subpart B of the FCC Rules for a Class A digital device, please note that this compliance is voluntary, for the instrument qualifies as an "Exempted device" under 47 CFR § 15.103(c), in regard to the cited FCC regulations in effect at the time of manufacture.

This instrument system is labeled, "For Research Use Only".

1. Introduction

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Meet the Chromo 4™ Continuous Fluorescence Detector

Thank you for buying a Chromo4 Continuous Fluorescence Detector from MJ Research, Incorporated. Designed by a team of molecular biologists and engineers, the Chromo4 detector will meet your needs for a sensitive, easy-to-use, and compact continuous fluorescence detection system. Some of the Chromo4 system's many features include:

- Designed to function like an Alpha™ unit compatible with our DNA Engine® thermal cyclers.
- Swappable with any Alpha unit, thus maintaining the flexibility of the cycler.
- DNA Engine Peltier thermal cyclers deliver superior thermal accuracy and well-to-well thermal uniformity.
- A 96-well sample block accepts standard consumables (96-well microplates and 0.2ml strip tubes).
- An integrated heated lid permits oil-free cycling.
- Long-lived LEDs excite fluorescent dyes.
- Sensitive photodiodes detect fluorescence.
- Opticon Monitor™ software facilitates experimental setup, run initiation, run-status monitoring, and data analysis.
- Two modes of temperature control: calculated control for maximum speed and accuracy; block control for adapting protocols optimized in other cyclers.

Using This Manual

This manual contains instructions for operating your Chromo4 system safely and productively:

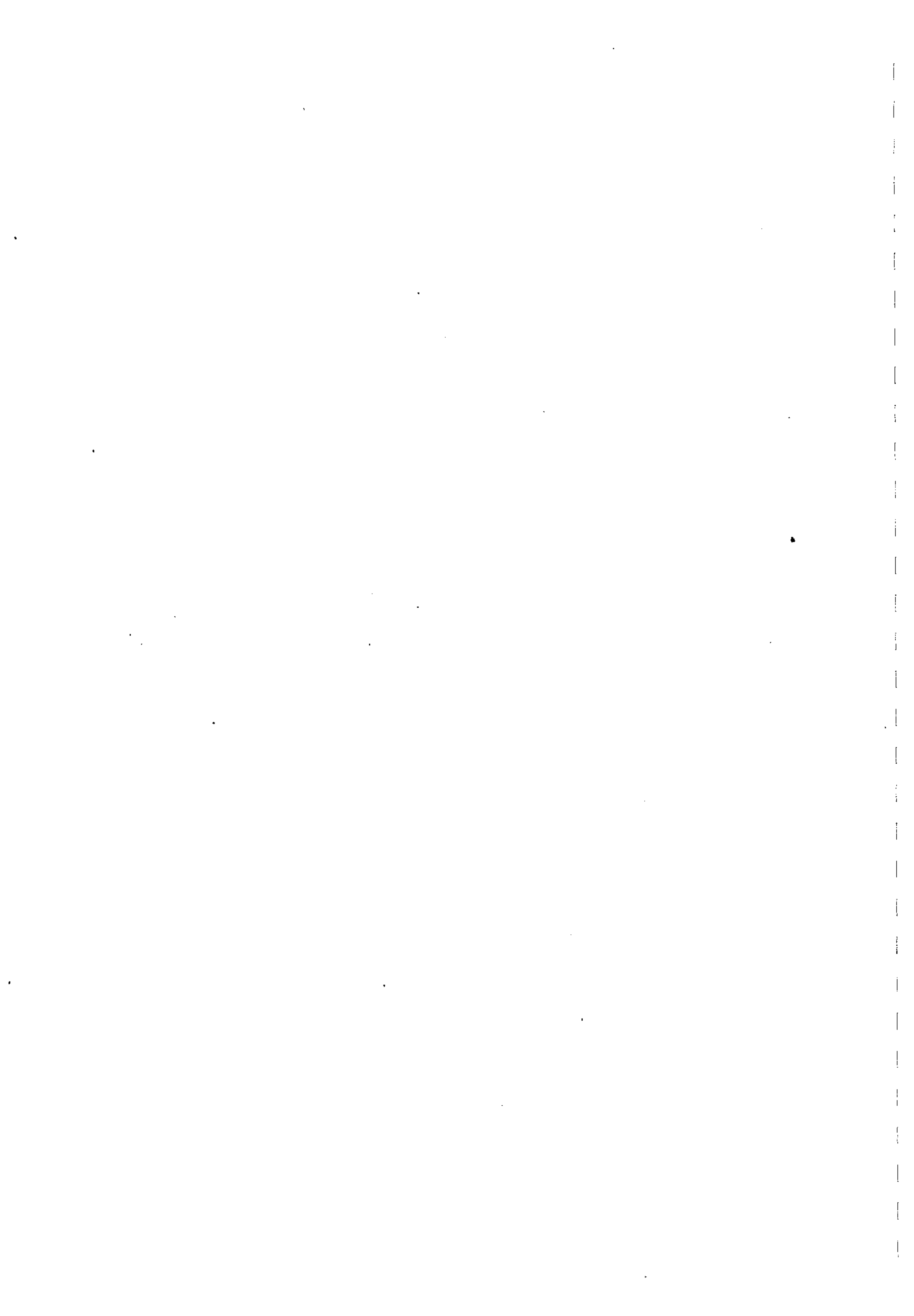
- Chapter 2 acquaints you with the **physical characteristics** of the Chromo4 detector.
- Chapter 3 presents the basics of **installing and operating** the Chromo4 detector.
- Chapter 4 discusses the **chemistry and sample vessel compatibilities** of the Chromo4 detector.
- Chapters 5-8 step you through the use of the **Opticon Monitor software** including how to enter and run protocols and how to analyze collected data.
- Chapter 9 explains the proper **maintenance** of the Chromo4 detector.
- Chapter 10 offers **troubleshooting** information for the Chromo4 detector.

Important Safety Information

Safe operation of the Chromo4 fluorescence detection system begins with a complete understanding of how the instrument works. Please read this entire manual before attempting to operate the Chromo4 detector.

The Chromo4 unit is designed to operate like an Alpha unit on a DNA Engine thermal cycler. In addition to reading this manual, please read *DNA Engine® & DNA Engine Tetrad® Peltier Thermal Cyclers, Operations Manual* to understand how that instrument works, before attempting to operate the Chromo4 detector in conjunction with the DNA Engine cycler. (The Engine manual was shipped with the thermal cycler.) Do not allow anyone who has not read these manuals to operate the instrument.

Warning: The Chromo4 system can generate enough heat to inflict serious burns and could deliver strong electrical shocks if not used according to the instructions in this manual. Please read the safety warnings and guidelines at the beginning of this manual on pages iv and v, as well as the warnings throughout the manual, and exercise all precautions outlined in them.



2. Layout and Specifications

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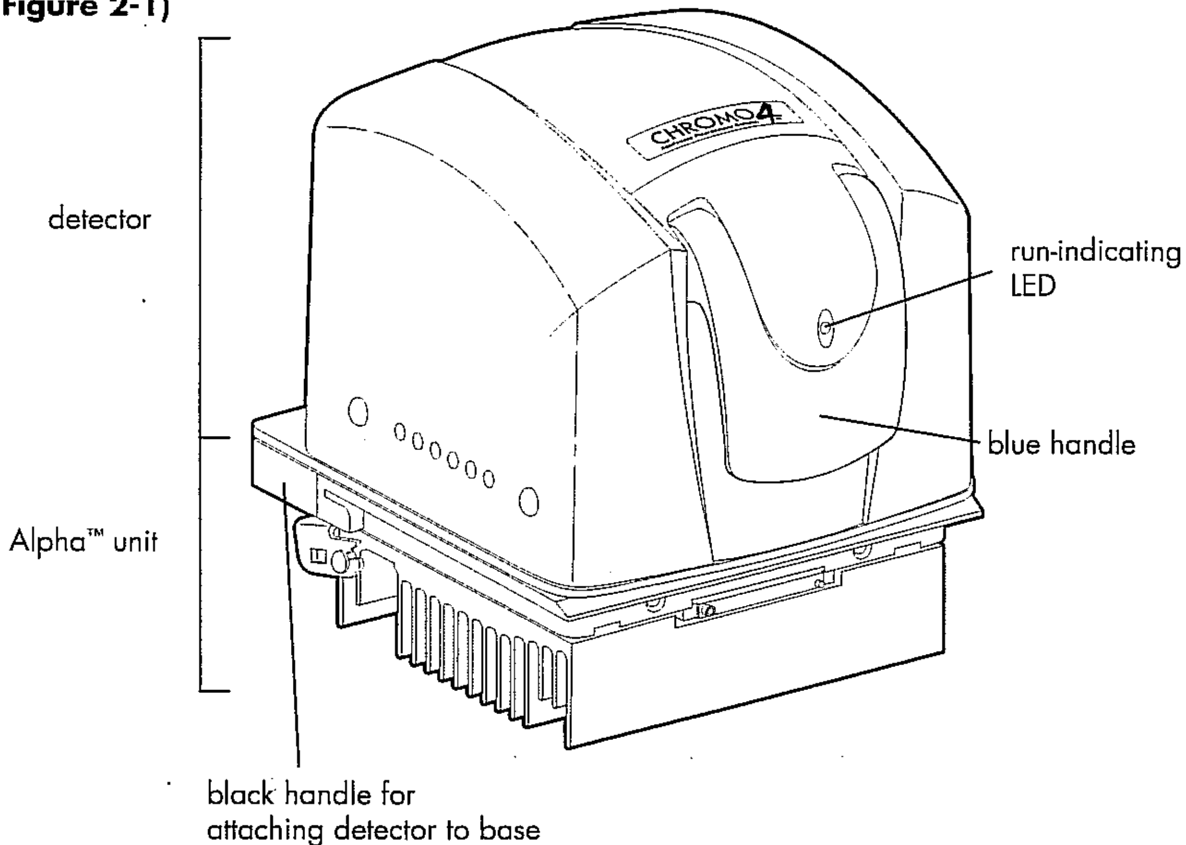
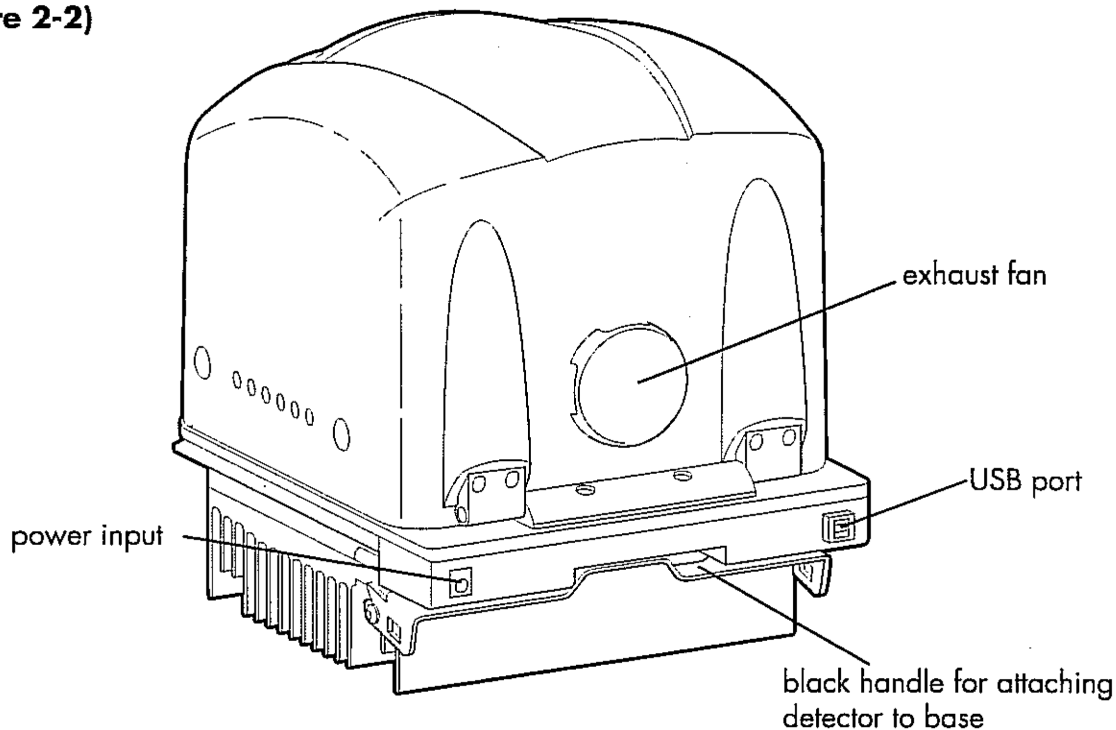
Front View
(Figure 2-1)



Back View
(Figure 2-2)



Specifications

Thermal range:	0° to 105°C, but not more than 30°C below ambient temperature
Thermal accuracy:	±0.3°C of programmed target @ 90°C, NIST-traceable
Thermal homogeneity:	±0.4°C well-to-well within 30 seconds of arrival at 90°C
Ramping speed:	Up to 3.0°C/sec
Sample capacity:	96-well microplate or 96 x 0.2ml tubes
Line voltage:	100-240VAC [1]
Frequency:	50-60Hz [1]
Power:	
thermal section:	40Vdc, 10A [2]
fluorescence section:	24Vdc, 2.5A [3]
Weight:	6.8kg (excluding thermal cycler, computer, and monitor)
Size:	23cm deep x 20cm wide x 18cm high (excluding thermal cycler, computer, and monitor)
Fluorescence Excitation Range:	Channel 1: 450-490nm Channel 2: 500-535nm Channel 3: 555-585nm Channel 4: 620-650nm
Fluorescence Detection Ranges:	Channel 1: 515-530nm Channel 2: 560-580nm Channel 3: 610-650nm Channel 4: 675-730nm

[1] No adjustment by the user is necessary to use electrical power within these ranges. However, all users should employ the proper IEC-320 cordset to connect the instrument to a power outlet, see page 3-4.

[2] The Chromo4 detection system is a component dedicated for thermal cyclers of the DNA Engine family, which supply power for the thermal section.

[3] Supplied by included power supply, Input: 100–240V ~1.5A 40–60Hz.

Gradient Specifications

Accuracy:	±0.3°C of target at end columns within 30 seconds (NIST-traceable)
Column uniformity:	±0.4°C, in column, well–to–well, within 30 seconds of target attainment
Calculator accuracy:	±0.4°C of actual column temperature (NIST-traceable)
Lowest programmable gradient temperature:	30°C
Highest programmable gradient temperature:	105°C
Gradient range: (temperature differential)	from 1.0°C up to 24.0°C

Computer Specifications

(minimum specifications required for running Chromo4 system and Opticon Monitor™ software version 2.03)

Operating System:	Windows 2000 or XP Pro
Processor Speed:	1GHz
Display:	1024 x 768 screen resolution
Memory:	256 MB RAM
Storage:	10GB hard drive

Specifications subject to change without notice.

3. Installation and Operation

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Unpacking the Chromo 4™ System

Note: Retain all packaging materials, undamaged, for use in the event of a service return.

1. Open the outer box (the "over-packing"), taking care not to damage it. Lift out the three inner boxes; these contain the Chromo4 unit, photonics shuttle (which contains the LEDs, photodiodes, filters, and electronics for excitation and detection), and accessory kit.

2. The smaller box at the top contains one or more white corrugated-cardboard photonics-shuttle boxes. Each photonics-shuttle box contains a dye-calibration CD-ROM matched to a specific photonics shuttle and a foam-filled plastic case containing the photonics shuttle itself. Note that the serial numbers on the CD-ROM, the photonics shuttle, and the plastic box should all match.

Note: Leave the photonics shuttle in the plastic box until you are ready to install it. Inside the box, the photonics shuttle is in a static-protective bag. Open this bag only when installing the photonics shuttle.

Caution: Never touch the circuit board on the back of the photonics shuttle: doing so could damage the electronics.

3. Remove the accessory kit from the inner box. Make sure all of the items listed under Packing Checklist (next page) are included.

4. Remove the Chromo4 unit together with the foam-plastic packaging end caps. Note that the Chromo4 unit is also in a static-protective bag. Remove the end caps.

5. Observing static-protective procedures, remove the Chromo4 unit from its plastic bag and proceed with installation.

Packing Checklist

The following items should have been shipped. If any item is missing or damaged, contact MJ Research immediately.

Chromo4 unit

Photonics shuttle box

- photonics shuttle
- dye calibration CD-ROM

Accessory kit

- 1 base-stabilizer bar
- 2 USB cables, A-B Male, 2 meters
- 1 serial interface cable, PC
- 1 power cord, black
- 1 box low-profile strip tubes (MJ catalog #TLS-0851)
- 1 box ultra-clear strip caps (MJ catalog #TCS-0803)
- 1 strip-cap seating tool (MJ catalog #ECT-2000)
- 1 power supply, 24V, 3.0A
- 1 Chromo4 alignment plate
- 1 USB-to-serial adapter
- 1 Opticon Monitor CD-ROM, version 2.03
- 1 Chromo4 Operations Manual (this document)

Conditions Required for Installing a Chromo 4 System

DNA Engine Requirements

To use a Chromo4 detection system, the DNA Engine® base (PTC-200) must be running version 3.3C or greater firmware. A previously purchased PTC-200 may need to be upgraded to version 3.3C or greater by following the instructions on page 3-14 of this document. To check the firmware version on a PTC-200 thermal cycler, perform the following steps:

1. Power on the PTC-200 (with an attached Alpha™ unit).
2. On the Main Menu programming screen, choose Setup using the select arrow keys.
3. Hit the Proceed key to advance to the next window.
4. Click Version on the programming screen using the select arrow keys.
5. Hit the Proceed key to advance to the next window.
6. Note the firmware version.

Note: A DNA Engine base that is running firmware versions 1.0A–1.1R cannot be upgraded (or become gradient enabled) without a hardware modification.

Computer Requirements

The minimum computer specifications required for Chromo4 system installation are as follows:

1. Operating system: Windows XP Pro or 2000
2. Memory: 256MB RAM
3. Processor speed: 1GHz
4. Storage: 10GB hard drive
5. Screen resolution: 1024 x 768pixels

Environmental Requirements

The Chromo4 system requires a location with four power outlets to accommodate the Chromo4 unit, the DNA Engine base, the computer, and the monitor. A location with network access (Ethernet 10/100BaseT) is recommended if you wish to transfer setup and analysis files between the computer running the Chromo4 system and other computers.

For reasons of safety and performance, ensure that the area where the Chromo4 system is installed meets the following conditions:

- Indoor use
- Nonexplosive environment
- Normal air pressure (altitude below 3000m)
- Ambient temperature 15°–31°C
- Relative humidity above 10% and up to 80%, non-condensing
- Installation Category II (portable equipment)
- Pollution Degree 2 Environment (normally only nonconductive pollution)
- Unobstructed access to air that is 31°C or cooler (see the following pages)
- Protection from excessive heat and accidental spills. (Do not place the Chromo4 system near such heat sources as radiators, and protect it from danger of having water or other fluids splashed on it, which can cause electrical short circuits.)

Power Supply Requirements

The Chromo4 system requires 100–240VAC, 50–60Hz and a grounded outlet. Supply voltage fluctuations should not exceed ±10%. The Chromo4 system can use voltage in the specified range without adjustment, so there is no voltage-setting switch.

The Chromo4 unit is equipped to accept power from an AC "brick type" adapter, which in turn can accept 100–240VAC, 50–60Hz. This adapter accepts cord sets with an IEC 60320-2-2 class II 2.5A 250V connector. Additionally, the cord set must meet all other applicable national standards—thus at a minimum, the cord set should carry the mark of a nationally recognized testing agency appropriate to your nation.

Note: Do not cut the supplied 120V power cord and attach a different connector. Use a one-piece molded connector of the type specified above.

Air Supply Requirements

A DNA Engine thermal cycler equipped with a Chromo4 unit requires a constant supply of air that is 31°C or cooler in order to remove heat from the detector's heat sink. Air is taken in from the vents at the front, back, and bottom of the cycler base and exhausted from the vents on both sides. If the air supply is inadequate or too hot, the instrument can overheat, causing performance problems and even automatic shutdowns.

Ensuring an Adequate Air Supply

- Do not block air intake vents.
- Position the cycler equipped with the Chromo4 unit at least 10cm from vertical surfaces and other thermal cyclers or heat-generating equipment (greater distances may be required; see the following section). Do not put loose papers under the base—they can be sucked into the air intake vents on the bottom of the instrument
- Do not allow dust or debris to collect in the air intake vents.

Ensuring That Air Is Cool Enough

- Do not position two or more cyclers (or other instruments) so that hot exhaust air blows directly into the air intake vents.
- Confirm that the cycler receives air that is 31°C or cooler by measuring the temperature of air entering the machine through its air intake vents.

Place the cycler equipped with the Chromo4 unit where you plan to use it, and turn it on. Try to reproduce what will be typical operating conditions for the machine in that location, particularly any heat-producing factors (e.g., nearby equipment running, window blinds open, lights on). Run a typical protocol for 30 minutes to warm up the system, then measure the air temperature at the air intake vents. If more than one instrument is involved, measure the air temperature for each.

If the air intake temperature of any instrument is warmer than 31°C, consult Table 3-1 for possible remedies. After implementing possible remedies, verify that the temperature of the air entering the air intake vents has been lowered, using the procedure outlined above.

Table 3-1 Troubleshooting Air Supply Problems

Cause	Possible Remedies
Air circulation is poor.	Provide more space around instrument or adjust room ventilation.
Ambient air temperature is high.	Adjust air conditioning to lower ambient air temperature.
Instrument is in warm part of room.	Move instrument away from, or protect instrument from, such heat sources as radiators, heaters, other equipment, or bright sunlight.
Instruments are crowded.	Arrange machines so that warm exhaust air does not enter intake vents.

Setting Up the Chromo 4 System

The Chromo4 unit is designed to be used like an Alpha unit on a DNA Engine thermal cycler. Please see the *DNA Engine® & DNA Engine Tetrad® Peltier Thermal Cyclers, Operations Manual,* which was shipped with your Engine, for complete instructions and requirements for setting up the thermal cycler.

Installing the Base-Stabilizer Bar on the DNA Engine Base

Important: Before installing the Chromo4 unit on the DNA Engine base, install the base-stabilizer bar on the rear, bottom of the base.

1. Tilt the cycler on its side so that the bottom air vents are accessible (Figure 3.1).
2. Orient the base-stabilizer bar so that the magnet faces the bottom of the cycler and the channel and bent tabs are toward the front of the cycler.
3. Insert the two bent tabs into the rearmost air vents.
4. Rotate the base-stabilizer bar toward the cycler until the magnet attaches to the cycler. The oval recess in the top of the stabilizer bar should fit over the plastic screw in the base of the cycler, and the back of the bar should extend approximately 7cm beyond the back of the cycler.

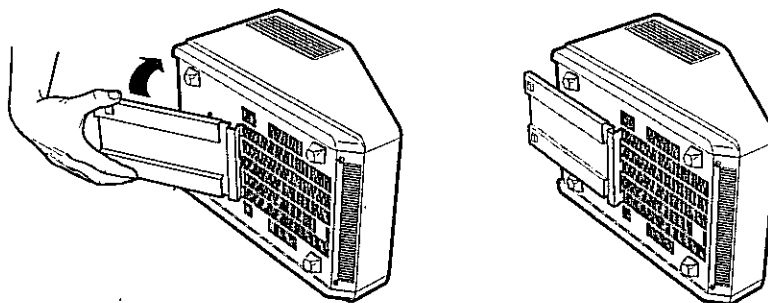


Figure 3.1. Installing the base-stabilizer bar on the DNA Engine base.

Installing the Chromo 4 Unit on the DNA Engine Base

1. Make sure the Engine base (the thermal cycler) is off.

Caution: Do not insert or remove the Chromo4 unit with the cycler turned on—electrical arcing can result. Read the safety warning at the beginning of this manual regarding electrical safety when inserting or removing the Chromo4 unit.

2. Hold the Chromo4 unit at its front and back edges.

3. Lower the Chromo4 unit into the DNA Engine base, leaving at least 3cm between the front edge of the Chromo4 unit and the front of the base.

4. Raise the black metal handle at the back of the Chromo4 unit and slide the unit forward as far as it will go (Figure 3-2a)

5. Push the back handle down until it is completely vertical and snug against the unit (Figure 3-2b)—firm pressure may be required. A definite click signals that the Chromo4 unit's connectors have mated with the cycler's connectors.

When the back handle is in the down position, the Chromo4 unit is locked into place.

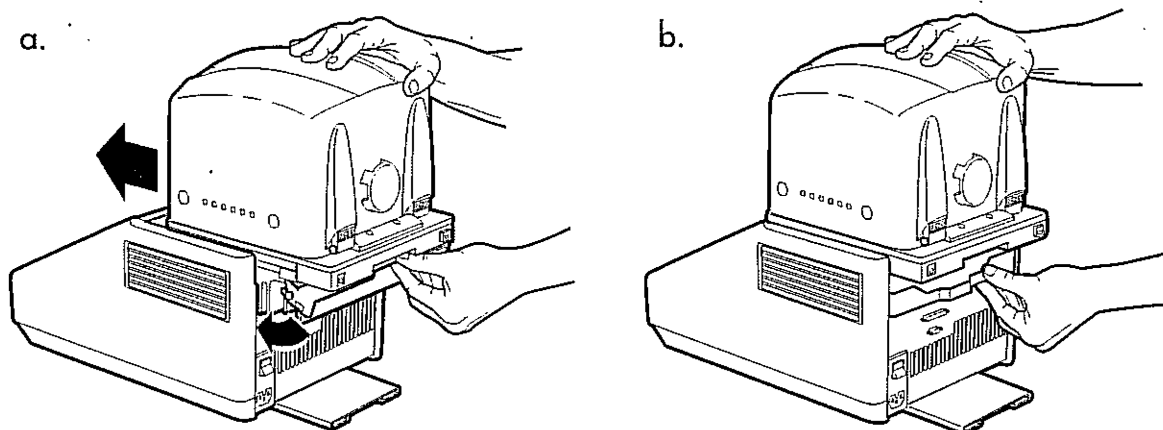


Figure 3-2. Installing the Chromo4 unit on the DNA Engine cycler.

Opening and Closing the Chromo 4 Detector

Be sure the base-stabilizer bar is attached to the cycler base before opening the lid of the Chromo4 unit.

Always use the blue handle to open and close the lid of the Chromo4 system. Improper opening and closing can damage the latch springs.

To gain access to the Chromo4 unit's sample holder, grip the bottom edge of the blue handle on the front of the lid and rotate the handle all the way upward (Figure 3-3). Now lift upward on the handle to open the lid of the Chromo4 unit, exposing the sample holder.

Note: Do not open the Chromo4 lid while the blue protocol-indicator light is on. Opening the lid, particularly during a scan of the plate, may interrupt the software's control of the protocol.

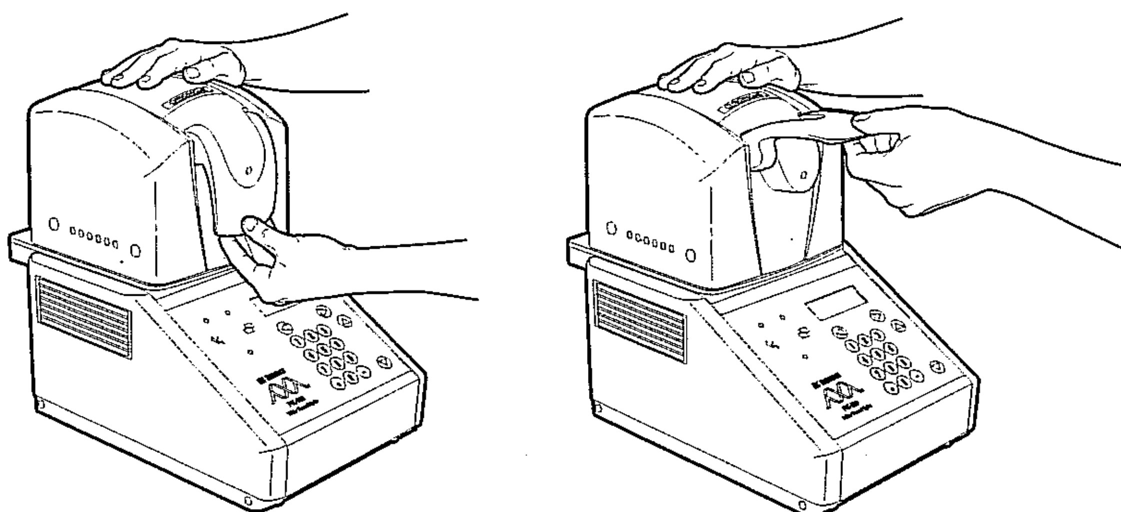


Figure 3-3. Opening the Chromo4 Detector.

To return the Chromo4 lid to the closed position, use the raised blue handle to lower the lid. When the lid is down, rotate the handle down and inward until it lies flush with the front panel that holds the blue indicator light.

Warning: Never attempt to force the Chromo4 housing closed with the blue handle in the down position. This puts unnecessary strain on the latch-spring mechanism, which may eventually cause the springs to fail.

Installing the Chromo 4 Photonics shuttle

The Chromo4 photonics shuttle contains the LEDs and photodiodes for illumination and detection, as well as appropriate filters and communication electronics. During plate reads, the photonics shuttle scans across the sample plate to interrogate each well.

Important: Before touching the photonics shuttle, discharge any static electricity that you may be carrying. One method of discharging static electricity, for example, would be to touch a grounded metal plumbing fixture shortly before touching the photonics shuttle.

Caution: Do not touch the circuit board on the back of the photonics shuttle: this could damage the electronics.

1. On the inner face of the Chromo4 lid is a metal heater plate with an 8 x 12 array of holes. The heater plate is held in place by two D-shaped latches along the anterior edge. Turn these latches to access the photonics -shuttle stage axes.

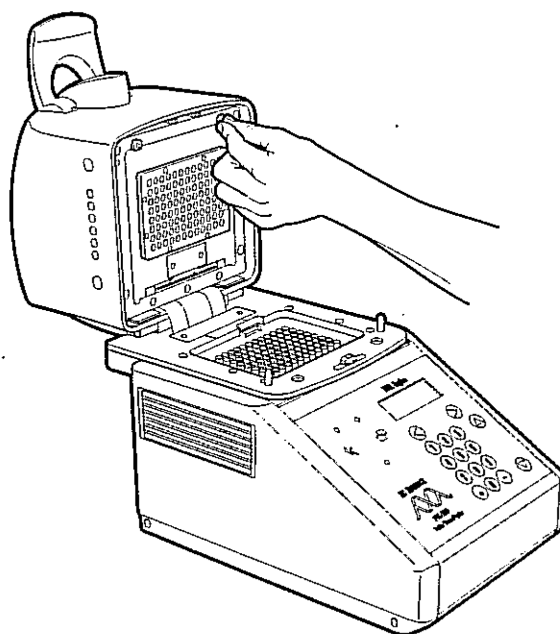


Figure 3-4. Accessing the photonics shuttle's stage.

2. When you first open the Chromo4 unit to install the photonics shuttle, you will see one red and one yellow plastic tube wrapped around the unit's stage axes. These protect the lead screws and prevent the photonics shuttle stage from moving during transit. **Remove these tubes and save them along with the other packaging.** Returning a unit without replacing these tubes may allow damage not covered by the warranty.

3. Center the shuttle stage by hand.

4. Remove the photonics shuttle from its plastic storage box and static-protective bag.

5. Orient the photonics shuttle so that when you insert it into the shuttle stage the circuit board will face the stage, the flattened pivot pins are at the bottom, and the metal side plates with holes to accommodate the ball detents are at the top. In this position, the 18-pin connector should insert into the connector on the shuttle stage.

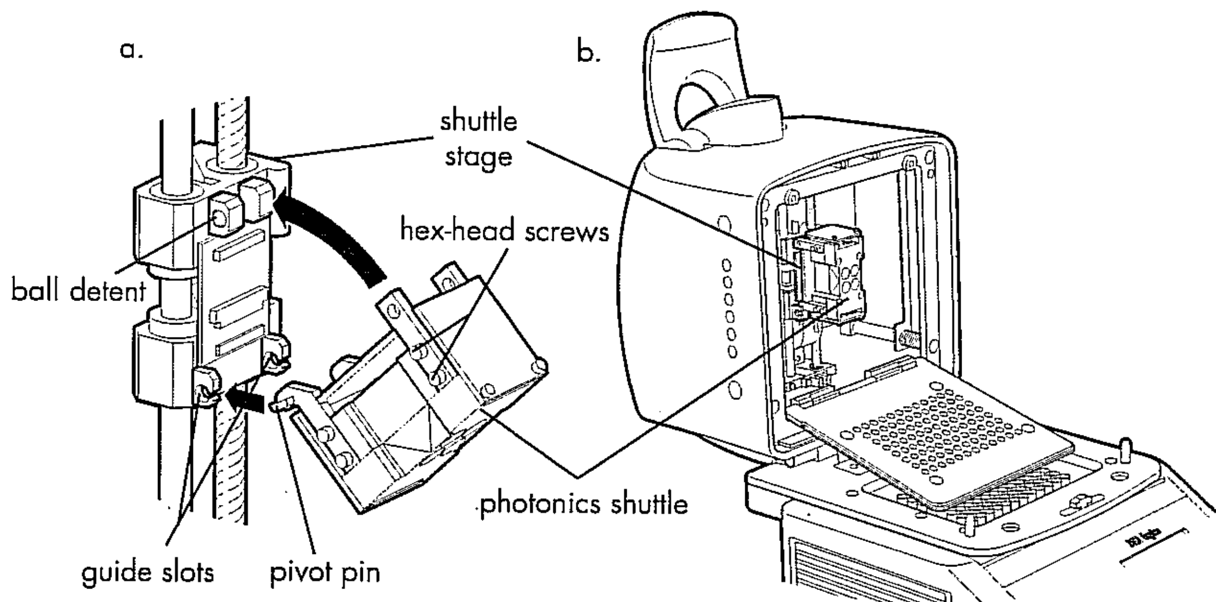


Figure 3-5. Installing the Chromo4 photonics shuttle.

6. Grasping the photonics shuttle between your thumb and forefinger, hold it at approximately a 60 degree angle to the stage's circuit board, so that the flattened pivot pins on the shuttle can slide into the guides on the stage.

7. Slide the pivot pins completely into the guides, then rotate the photonics shuttle upward, pressing it against the stage plate until it snaps into the ball detents on the stage.

8. Close the heater plate and rotate the latches to hold the plate in place.

Attaching the Power Cords and Communication Cables

Power Connection

1. Insert the cycler power cord plug into its jack at the back of the base, and then plug the cord into an electrical outlet (see *DNA Engine® & DNA Engine Tetrad® Peltier Thermal Cyclers, Operations Manual* for more details).

2. Insert the switching power supply plug into its jack at the left bottom side of the Chromo4 unit, and plug the other end into a standard 110V or 220V electrical outlet.

Turning the Chromo 4 Detection System On and Off

The Chromo4 unit does not have its own power switch; it will receive power when it is plugged in, and will be controlled by the Opticon Monitor software.

The power switch for the DNA Engine thermal cycler is located on the back of the unit, just above the power cord. To turn the cycler on, press the switch so that the side marked "I" is depressed. The red power-indicator light on the base will turn on. The blue indicator light on the Chromo4 unit will not be illuminated until a protocol is running.

The thermal cycler requires several minutes to warm up after it is powered up.

To turn the cycler off, depress the "0" side of the power switch.

Serial Connection

Note: *When the serial cable is attached to the DNA Engine thermal cycler, the front panel of the cycler is nonfunctional. Instead, the cycler is controlled by the computer, using Opticon Monitor software.*

1. Turn on the power to both the computer (with no peripherals plugged in) and the DNA Engine base with the Chromo4 detector installed.

2A. If the computer has a serial port, first connect the serial cable to the PTC-200, and then connect the serial cable to the computer. Note the COM port number into which the serial cable has been inserted, as it will be used in the machopts file when installing Opticon Monitor software.

2B. If the computer does not have a serial port, use the serial-to-USB adapter. Instructions are as follows:

a. Plug the provided USB-to-serial adapter into a USB connection on the computer (do not plug in the serial cable from the PTC-200 yet). The blue light on the adapter should illuminate.

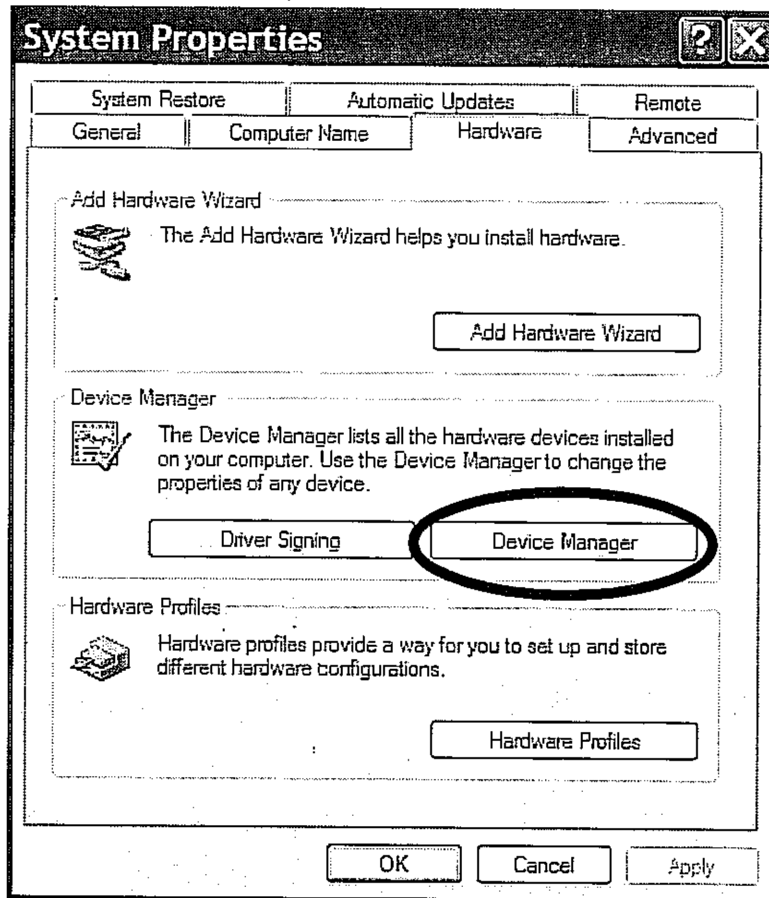
b. The Windows OS will open the Found New Hardware Wizard.

c. Insert the CD provided with the adapter and follow the driver installation instructions. You will receive a warning that the software has not passed Windows Logo testing to verify its compatibility with Windows XP. Click the Continue Anyway button to proceed.

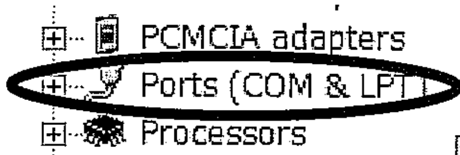
d. The Found New Hardware Wizard will indicate when the software installation is complete. Click Finish to close the Wizard.

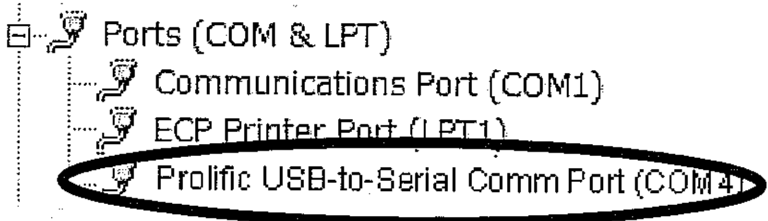
e. Plug the serial cable from the PTC-200 into the adapter. The blue LED on the adapter should get brighter.

f. Document the COM port number into which the USB-to-serial adapter has been inserted by right clicking on "My Computer" and selecting "Properties". Then, click the "Hardware" tab and the following window will appear:



g. Click on "Device Manager" and then select "Ports (COM & LPT) as shown below, left. The device along with a COM port designation should be present (Note, the Cables To Go adaptor will be listed as "Prolific USB-to-Serial Comm Port" as shown below, right). You will need to record the COM port number shown in parenthesis ('4' in the figure below) in the machopts file during Opticon Monitor installation.





USB Connection

1. Copy the folder entitled MJ USB Driver from the Chromo4 Dye File Disk onto the computer desktop. This folder contains the files MJR.inf, MJRdetec,sys, MJRQuad.sys, and quadldr.sys.

2. Plug the USB cord into the Chromo 4 detector and then into the computer.

3. The Windows OS will pop up a device message alerting the user it detects an MJ Real-Time Detector. The New Found Hardware Wizard will then open.

4. Select "Install from a list or specific location (Advanced)". Browse for the MJ USB Driver folder on the desktop.

5. Follow the Wizard instructions. A warning will appear indicating the MJ Real-time Detector USB Device has not passed Windows Logo testing. Click the Continue Anyway button to proceed.

Upgrading DNA Engine Firmware (if necessary)

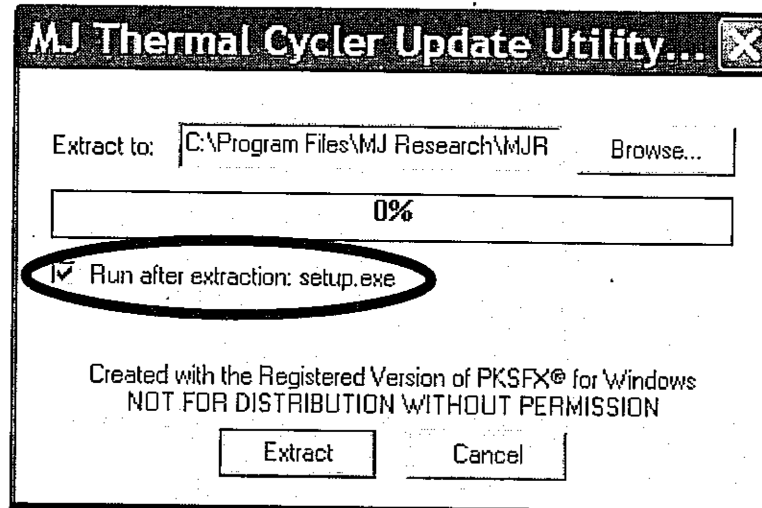
Once the serial connection between the thermal cycler and the computer has been established, the DNA Engine firmware can be upgraded to version 3.3C, if necessary, by following the instructions below.

1. Download the DNA Engine Update Utility and the latest firmware version from www.mjr.com. The two current versions are MJ Instrument Updater (MJ Thermal Cycler Update Utility 1_3.EXE) and 3.3C files (33CEngine.EXE). Icons for these programs are shown below:

33CEngine.EXE
PKSFX® for Windows
PKWARE, Inc.

MJ Thermal Cycler Update Utility
1_3.EXE
PKSFX® for Windows

2. First install the MJ Updater, by double clicking on the MJ Thermal Cycler Update Utility 1_3.EXE icon. In the next window, check the box for "run after extraction: setup.exe" as shown below. Click the Extract button to run the installer.



3. When extraction is completed, the program prompts to run the setup.exe program. Click the Yes button.

4. Follow the instructions for the InstallShield Wizard for MJ Thermal Cycler Update utility. This procedure will drop the following shortcut icon on the desktop:

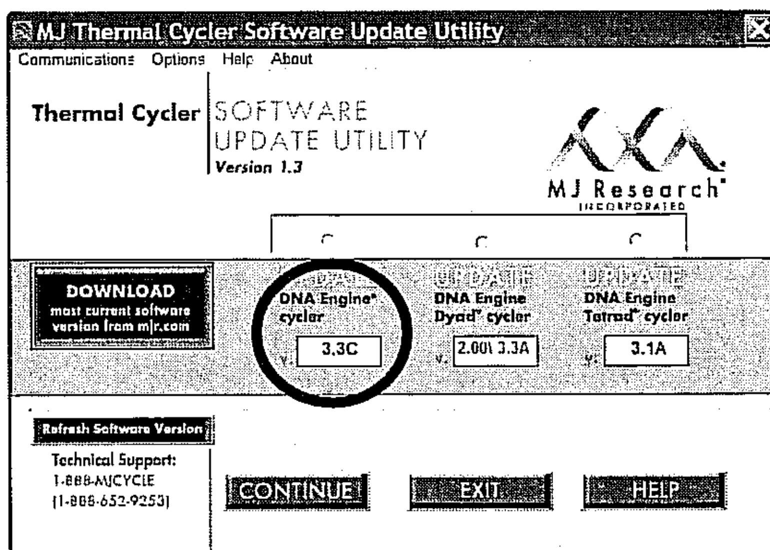
MJ Thermal Cycler Update Utility.exe

5. After installing the updater utility, open and extract the 3.3C file. Double click on the 33CEngine.EXE icon. In the next window, check the box for "run after extraction: setup.exe" and click the Extract button.

6. Run the MJ Instrument Updater by clicking on the shortcut icon on the desktop. A "failure to initialize serial port" warning will be registered. Click Ok.

7. Instructions will indicate that a serial port has not been selected. Click Ok.

8. The software update window shown below will open.



9. Under "Communications", select the correct serial cable COM port number (see section on Serial Connection, on page 3-12, for instructions).

10. Select Update DNA Engine cycler and verify that the program lists firmware version 3.3C.

11. Click the Continue button to update the PTC-200 firmware.

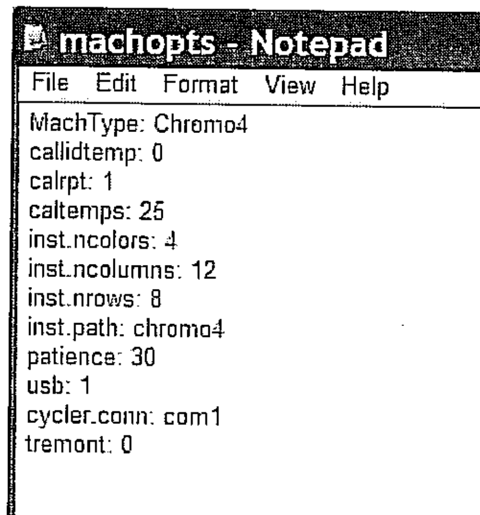
12. Follow the instructions given from the updater (read instructions carefully).

13. After the update, a message will prompt you to turn the thermal cycler power off and then restart it. Do so, and then press Exit.

Installing Opticon Monitor™ Software

1. Install the Opticon Monitor 2.03 installer from the disk provided by inserting the disk and double-clicking on the installer icon. Follow the installation instructions. Reboot the computer.

2. Go to C:\Program Files\Opticon Monitor 2\instrument\chromo4 and open the machopts file with Notepad. The file should look like this:



3. If necessary, change the COM port number in the machopts file, listed above as "cycler.conn: com1", to the COM port number in which the serial cable has been inserted (from section on Serial Connection, page 3-12).

Warning: Do not change any other parameters in the machopts file. Doing so could damage the instrument and void the warranty.

4. Save the machopts file.

Copying the Dye Files

Insert the Chromo4 dye files CD-ROM and copy the Chromo4 dye calibration files to

C:\Program Files\Opticon Monitor 2\Instrument\Chromo4\Dyes

Aligning the Chromo 4 Photonics shuttle

1. Open Opticon Monitor v.2.03 software.
2. Make sure 'Chromo4' is the selected instrument in the Instrument panel. Note, 'Chromo4' should be the only instrument listed.
3. Under the *Tools* menu select *Change Chromo4 Photonics shuttle.*
4. A window will appear. Select Yes and follow instructions to align the photonics shuttle using the provided alignment plate.

Changing the Chromo 4 Photonics shuttle

Important: Before touching the photonics shuttle, discharge any static electricity that you may be carrying. One method of discharging static electricity, for example, would be to touch a grounded metal plumbing fixture shortly before touching the photonics shuttle.

Caution: Do not touch the circuit board on the back of the photonics shuttle: this could damage the electronics.

1. Unplug the power from both the Chromo4 unit and the DNA Engine thermal cycler.

2. Open the lid of the Chromo4 detector. Be careful—it may be warm.
3. Open the Chromo4 housing by turning the D-shaped latches along the anterior edge that hold the heater plate in place (Figure 3-4). This will remove power from the instrument—you may receive a USB-detach warning from the Windows OS.
4. When the Chromo4 housing is open, the photonics shuttle's filters will be visible (Figure 3-5b). To remove the photonics shuttle, grasp it firmly between your thumb and forefinger along its upper edges, where there are two hex-head screws. Pull on the photonics shuttle, rotating it outward on the flat pivot pins until the pins can be lifted free of their guide slots on the photonics shuttle stage (Figure 3-5a).

Note: Every time you remove and replace the photonics shuttle, you must realign the detector (see page 3-17) using the alignment plate that came with the instrument. The last screen of the *Change the Photonics shuttle* protocol instructs you to insert the alignment plate.

Loading Sample Vessels into the Detector

To ensure uniform heating and cooling of samples, sample vessels must be in complete contact with the sample holder. Adequate contact is ensured by doing the following:

- Ensure that the sample holder is clean before loading samples (see Chapter 9 for cleaning instructions).
- Firmly press strips of 0.2ml tubes, or a 96-well microplate into the sample-holder wells (see the "Selecting the Correct Sample Vessel" section in Chapter 4).
- MJ Research strongly recommends that oil **not** be used to thermally couple sample vessels to the holder.

Tip: Air bubbles in samples, or liquid on the plate deck, can adversely affect results in real-time experiments. You may wish to spin down reactions in tubes or microplates to remove any air bubbles before loading the vessels into the thermal-cycler sample holder.

4. Compatible Chemistries, Sample Vessels, and Sealing Options

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Optical System

The Chromo4™ system uses a scanning photonics shuttle containing four LEDs and four photodiodes to sequentially illuminate and detect emitted fluorescence from each of the 96 wells in the cycler block.

The Chromo4 detector is calibrated at the factory and requires no further calibration before use. See Chapter 10, "Troubleshooting" for instructions on how to test the detector's calibration and how to recalibrate, if required.

Compatible Chemistries

The Chromo4 system is compatible with popular dye chemistries including SYBR Green I, molecular beacons, hydrolysis probes (TaqMan probes), Scorpions probes, the Amplifluor system, and LUX Fluorogenic Primers. In addition to performing real-time quantification* and DNA melting profiles, the DNA Engine cycler configured with a Chromo4 detector is useful as a temperature-controlled fluorimeter for a number of applications including ligand binding and protein structure studies. If you have questions regarding the compatibility of a particular chemistry with the Chromo4 detector, contact MJ Research technical support at 888-652-9253.

SYBR Green I (SGI)

SYBR Green I (available from Molecular Probes, Inc. of Eugene, Oregon) is a double-stranded-DNA binding dye. Because the fluorescence of SGI is greatly enhanced upon binding dsDNA, it is an ideal dye for the detection of amplification products. The maximum absorbance of SGI is ~497nm and the emission maximum is ~520nm[1].

SGI offers several advantages for detection of nucleic acids in real time. Because SGI binds to all dsDNA, it does not have to be customized for individual templates. Therefore, protocols can be adapted quickly and at relatively low cost. Furthermore, because multiple SGI molecules bind to a single amplification product, SGI is very sensitive. On the other hand, because SGI binds to all dsDNA, false positive signals from primer-dimers, secondary structure, or spurious priming can interfere with accurate quantification. Measuring fluorescence at elevated temperatures may help reduce the detection of nonspecific products[2]. Performing a melting curve analysis can help to identify optimal annealing and plate-read temperatures, to avoid generating false positives. Melting curve analysis can also be used to analyze product homogeneity after a run.

MJ Research recommends using buffers containing 5% dimethyl sulfoxide (DMSO) with a concentration of 1X or less SYBR Green I with the Chromo4 detector. For additional information on optimizing protocols using SYBR Green I with thermostable enzymes available from MJ Research, contact MJ Research technical support at 888-652-9253.

[1]Molecular Probes, Inc.

[2]Morrison, T.B., J.J. Weis and C.T. Wittwer. 1998. Biotechniques 24:954-962.

*The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche, exclusively licensed to Applied Biosystems. Use of the PCR process requires a license.

Molecular Beacons

Molecular beacons are dual-labeled oligonucleotide probes designed to form stem-loop structures in the absence of target. In the hairpin configuration, the fluorophore at one end of the molecule is brought into close proximity with a quenching moiety at the other end of the molecule. When the fluorophore is excited in this configuration, it transfers energy to the quencher rather than emitting that energy as light, in a process known as fluorescence resonance energy transfer (FRET). A "dark" quencher is often used, so the energy transferred from the fluorophore is emitted in the infrared, rather than the visible range. If a second fluorophore is used as a quencher, the transferred energy is emitted as light at the quenching fluorophore's characteristic wavelength.

Molecular beacons are designed such that the loop, which is usually 15–30 nucleotides long, is complementary to the target sequence. The arms flanking the loop, which are usually 5–7 nucleotides long, are designed such that they are complementary to each other, and thus favor formation of a stem structure. A fluorophore is attached to the end of one arm, and a quencher is attached to the other. Molecular beacons must be carefully designed so that, at the annealing temperature of the reaction, hairpins form in the absence of template but annealing of the loop sequence to the target is energetically favorable in the presence of template. When the loop of a molecular beacon hybridizes to the target sequence, the conformational change of the probe separates the fluorophore and the quencher. When the fluorophore is excited, it now emits light at its characteristic wavelength.

One advantage of molecular beacons is that, unlike SYBR Green, molecular beacons specifically detect the target of interest. Great sensitivity, including detection of single nucleotide polymorphisms (SNPs), is possible with carefully designed molecular beacons under optimized reaction conditions (temperature, buffer). The major disadvantage is that each probe must be carefully and uniquely designed for the detection of a specific target.

Molecular beacons are a technology patented by the Public Health Research Institute of New York, NY, and are available from a number of licensed suppliers. When designing molecular beacons for use with the Chromo4 detector, fluorophores with excitation and emission spectra falling within one of the Chromo4 detector's excitation and detection range pairs, such as FAM, should be used. Either dark quenchers or a quenching fluorophore may be used with the Chromo4 detector.

Hydrolysis Probes (TaqMan Probes)

TaqMan probes are a patented technology available from a number of licensed suppliers. They are oligonucleotide probes whose fluorescence is dependent on the amplification of a target sequence. TaqMan probes are designed to anneal to the target sequence, between the forward and reverse primer-binding sequences. A reporter fluorophore is attached to the 5′ end of the probe and a quencher to the 3′ end.

When the intact probe anneals to the target sequence (and when it is not bound), excitation of the reporter is quenched because of its proximity to the 3′ quencher. However, as extension proceeds, the 5′ exonuclease activity of the polymerase cleaves the probe, separating the reporter from the quencher. TaqMan probes work well with enzymes derived from *Thermus* species, such as DyNAzyme™ II DNA polymerase from *Thermus brockianus*, available from MJ Research, Inc. Liberated reporter molecules accumulate as the number of cycles increases, so the increase in fluorescence is proportional to the amount of amplified product.

One advantage of TaqMan probes, particularly for quantification, is that fluorescence is dependent not only on the presence of a specific target, but also on amplification of that target. Like molecular beacons, however, TaqMan probes must be individually designed for specific targets. See the "Molecular Beacons" section above for recommendations on the use of specific fluorophores and quenchers with the Chromo4 detection system.

Scorpions Probes

Scorpions probes (available from licensed suppliers) contain an amplification primer linked, through an intermediary portion containing an amplification stopper, to a target-specific probe. The sequences flanking the probe sequence are complementary to each other, and can form a hairpin structure. This hairpin structure brings a fluorophore and a quencher into close proximity.

During amplification, extension of the target sequence proceeds from the primer portion of the Scorpions probe. During denaturation, the hairpin loop opens. As the reaction cools following denaturation, the target-specific Scorpions probe sequence binds to the amplified, complementary target sequence, so the original hairpin structure does not re-form. Thus the fluorophore and quencher are separated, and fluorescence emission can be detected. The amplification stopper prevents read-through during amplification, which could lead to opening of the hairpin, and thus fluorescence detection, in the absence of target.

Because the Scorpions probe is integrated into the product, there is a direct relationship between the number of targets generated and the amount of fluorescence.

See the "Molecular Beacons" section above for recommendations on the use of specific fluorophores and quenchers with the Chromo4 detection system.

Amplifluor Universal Detection System

The Amplifluor system (available from Intergen Company of Purchase, NY) makes use of a universal primer that emits a fluorescence signal only following incorporation of the primer into an amplification product. The universal primer consists of an 18-base primer tail ("Z sequence") coupled to a hairpin sequence labeled with a fluorophore and a quencher. First, the target is amplified using target-specific primers, one of which has the Z sequence added to its 5' end. In the following round of amplification, the complement to the Z sequence is incorporated into the product. The universal primer then anneals to the complement of the Z sequence and extension proceeds. In the next cycle, extension

proceeds through the universal primer, incorporating it into the amplification product. In the process, the hairpin is unfolded, separating the fluorophore and quencher and emitting a fluorescence signal that is proportional to the amount of amplified product.

See the "Molecular Beacons" section above for recommendations on the use of specific fluorophores and quenchers with the Chromo4 detection system.

LUX Fluorogenic Primers

LUX (Light Upon eXtension) Fluorogenic Primers (available from Invitrogen Corporation, Carlsbad, CA) come as a primer set. One primer is labeled with a single fluorophore, while the other primer is unlabeled. The labeled primer is designed to form a hairpin loop that causes an intrinsic quenching of the fluorophore. When the primer binds to the target and extension begins, the primer is incorporated into the double stranded PCR product and the fluorescence emitted by the fluorophore increases. These primers are sensitive and target specific, and allow melting curve analyses to be performed. Although the primers have to be designed specifically for the target of interest, there is no requirement for additional probes, as there is with the TaqMan system.

See the "Molecular Beacons" section above for recommendations on the use of specific fluorophores and quenchers with the Chromo4 detection system.

Selecting the Correct Sample Vessel

For best results, use thin-wall microplates or 0.2ml tubes with white or clear wells with the Chromo4 system. Some users choose vessels with opaque, white wells to achieve higher signal strength in fluorescence-detection assays. Other users prefer vessels with clear wells, for easier sample viewing. MJ Research offers both microplates and tubes with either opaque, white wells or clear wells. Our vessels are designed for fluorescence detection assays and are optimized to precisely fit in the cycler block (see the "Sample Vessel and Sealing Selection Chart for Optical Assays" starting on page 4-7).

Microplates with black wells may be useful in applications requiring very low levels of background, but signal strength is significantly reduced when plates and tubes with black wells are used.

Note: In-factory calibration of the Chromo4 detector is performed with both clear and opaque white microplates.

Sealing Sample Vessels

To achieve accurate, uniform, and repeatable results in thermal-cycling reactions, proper sealing of vessels is at least as important as high light transmission. Tight sealing is necessary to prevent evaporative losses and resulting increases in the concentration of reaction components. Only a layer of mineral oil or wax, such as Chill-out liquid wax, will completely prevent evaporation from sample vessels. Nonetheless, an adequate degree of protection can be achieved by sealing with optical caps or sealing film (in conjunction with an optical compression pad—see below), and cycling the samples using the Chromo4 unit's heated lid to prevent condensation.

Sealing with Optical Caps or Sealing Film and the Heated Lid

Excellent sealing of sample vessels can be achieved with optical caps or sealing film when used with the Chromo4 unit's heated lid. The heated inner lid maintains the upper part of sample vessels at a higher temperature than the reaction mixture, thus inhibiting condensation of evaporated water vapor onto the vessel walls, and leaving solution concentrations unchanged during thermal cycling. The heated lid also exerts pressure on the tops of vessels loaded into the block, which is necessary to maintain a vapor-tight seal.

Ultra-clear, optical strip caps available from MJ Research provide high light transmission for fluorescence detection and reliable, vapor-tight sealing.

The Optical Film Sealing Kit from MJ Research includes optically clear, adhesive seals and a compression pad that improves sealing. The excellent sealing capabilities and high light transmission of this kit have produced more uniform results and earlier C(t) values than other optical films that appear more transparent.

Notes for use:

- When tubes are cooled to below ambient temperatures, a ring of condensation may form in tubes above the liquid level but below the top of the sample block. This is not a cause for concern since it occurs only at the final cool-down step, when thermal cycling is finished.

Sealing with Chill-out™ Liquid Wax

Clear Chill-out liquid wax (available from MJ Research) provides 100% prevention of condensation and vapor loss and provides excellent light transmission for optimal performance in optical assays. At room temperature and above, this overlay is a transparent liquid and can be applied by pipette. The wax solidifies below 10°C, thus protecting samples during cold storuage. The solidified wax is easily pierced with a pipette tip, so the aqueous sample is easily retrieved, if needed.

Notes for use:

- Use 15µl of Chill-out liquid wax for a 20µl reaction, 20µl for a 50µl reaction.
- Include the volume of wax in the total volume when setting up a calculated-control protocol.
- Be sure to use the same amount of wax in all reaction wells, to ensure a uniform thermal profile.

Sample Vessel and Sealing Selection Chart for Optical Assays

The following reaction vessels and sealing options are recommended for use with the Chromo4 system and are available from MJ Research. To place an order, call 888-729-2165 or fax 888-729-2166.

Product	MJ Research Catalog Number	Product highlights
Vessels		
0.2ml tubes, strips of 8	TLS-0851 low profile, white TLS-0801 low profile, clear TBS-0201 full height, clear	• Ideal for small numbers of samples • Use white wells for maximum signal, clear wells for easier sample viewing. • Use low profile vessels for reaction volumes ≤25µl.
Hard-Shell® 96-well skirted microplates	HSP-9655 white shell, white wells HSP-9601 white shell, clear wells	• Ideal for 96 samples • Plate remains absolutely flat during thermal cycling, for uniform light collection • Use white wells for maximum signal, clear wells for easier sample viewing. • Colored shells are available for color-coding.
Multiplate™ 96-well unskirted microplates	MLL-9651 low profile, white MLL-9601 low profile, clear MLP-9651 full height, white MLP-9601 full height, clear	• Good for fewer than 96 samples —microplates can be cut to size • Pre-cut, 48- and 24-well microplates are also available. • Use white wells for maximum signal, clear wells for easier sample viewing. • Use low profile vessels for reaction volumes ≤25µl.

Product	MJ Research Catalog Number	Product highlights
Sealing Options		
Optical flat caps, strips of 8	TCS-0803	• Ultra-clear caps yield high light transmission and robust sealing • For thermal-cycling volumes ≥5µl
Optical Film Sealing Kit	MSO-1001	• Clear, adhesive seals yield optimal light transmission and strong sealing for consistent results. • Includes optional compression pad; additional pads can be ordered separately (ADR-3296). • For thermal-cycling volumes ≥10µl • Can be used for plate storage, ambient to –40° C
Chill-Out™ Liquid Wax, optical-grade	CHO-1411	• Replaces mineral oil overlay • Provides high light transmission • For thermal-cycling volumes ≥2µl

Reaction Volume Recommendations

Reaction volumes of 20µl are recommended for most applications. Volumes as low as 10µl can be used, although sensitivity may be slightly reduced and variability slightly increased. The maximum recommended sample volume is 100µl. Volumes exceeding 100µl do not maintain adequate contact with the wells of the sample block, and can result in nonuniform heating and cooling within the sample. It is beneficial to empirically optimize reagent concentrations and sample volumes with the Chromo4 system, because the sensitivity of the optical system often allows a cost-saving reduction in reagent concentrations.

Thermal accuracy is optimum when all samples contain identical volumes and this volume is used to calculate the temperature of the samples during a calculated-control run (see the "Temperature Control Method" section in Chapter 6).

5. Introduction to Opticon Monitor™ Software

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Introduction

Opticon Monitor software controls the Chromo4 Continuous Fluorescence Detection System and performs data analysis. This chapter will introduce you to Opticon Monitor software and discuss the basics of launching and navigating the software. Chapter 6 describes experimental setup and programming, Chapter 7 discusses run initiation and status, and Chapter 8 focuses on data analysis. **This manual documents version 2.03 of the Opticon Monitor software.**

Installing Opticon Monitor Software

Opticon Monitor software is compatible with Windows 2000 and Windows XP Pro operating systems. Users may install the software on multiple computers, and data analysis may be performed on computers not attached to the Chromo4 system.

To install Opticon Monitor software, insert the program disk into the computer's CD drive. The Install Wizard should automatically open when the computer reads the disk. If the Install Wizard does not open, view the disk's contents and double-click on the Opticon Monitor icon.

To complete installation, follow the instructions on the Install Wizard screens.

Note to users of previous versions of Opticon Monitor software: If Opticon Monitor is already installed on the computer, the Install Wizard will first uninstall the existing version. You then must run the Install Wizard a second time to install Opticon Monitor version 2.03.

Using the Install Wizard on the Opticon Monitor program disk to uninstall Opticon Monitor will **not** affect previously stored data, master, plate, or protocol files. To avoid deleting important files, use the Install Wizard on the Opticon Monitor disk, as described above, to uninstall the Opticon Monitor program.

Caution: Using Windows software to uninstall Opticon Monitor will erase all Opticon Monitor files, including data files.

How Opticon Monitor Software Works

Opticon Monitor software is structured such that different types of operations are accessed from different screens. Operations can be categorized into three stages: experimental setup, data acquisition and monitoring, and data analysis.

1. Experimental setup and programming. Setup and programming operations are accessed from the Master File window. The master file consists of a plate file, which specifies the parameters (e.g. well contents) of the plate, and a protocol file, which specifies the experimental conditions (e.g. incubation temperatures and timing of fluorescence measurements). Users can create new files or use existing files, in their current form or after editing.

2. Run initiation and status. After a plate and a protocol setup have been created or selected, a run can be initiated. The user may stop the run at any time and may skip protocol steps, if desired. The Status window appears automatically upon run initiation and can be used to monitor the progress of the run and the ongoing thermal profile of the sample, block, and/or lid. Data collection can be monitored during the run by plotting fluorescence intensity vs. cycle number.

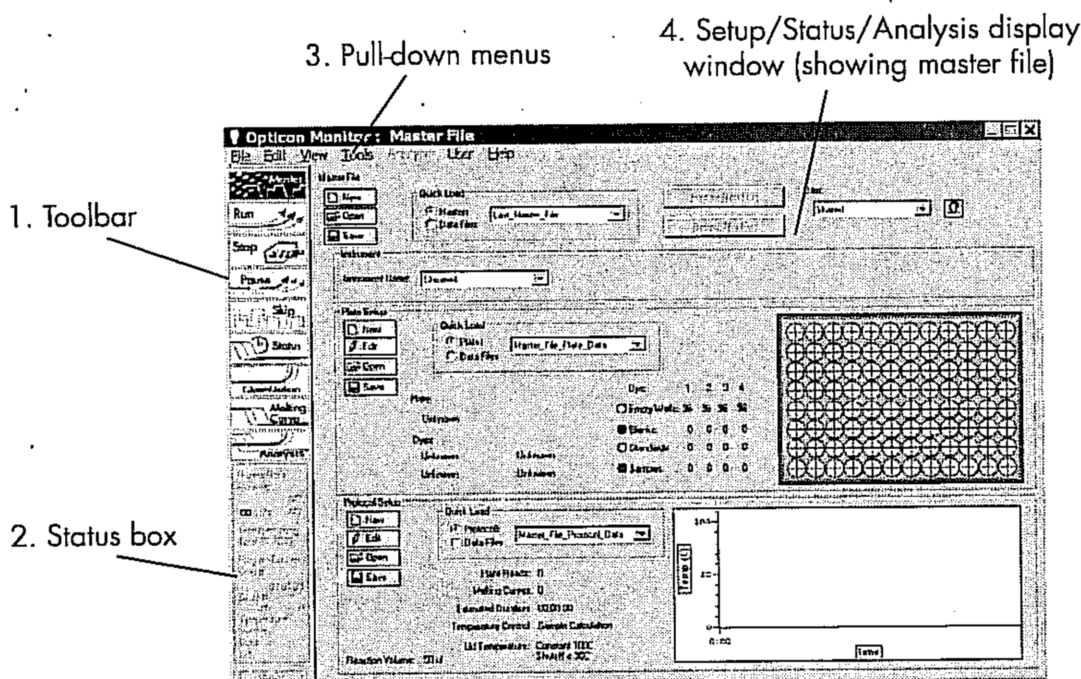
3. Data analysis. Depending on the type of data being collected (as specified in the master file) data can be analyzed using the Quantitation, Melting Curve, and/or Analysis windows. Starting copy number can be quantified either during or after the run by using the functions in the Quantitation window to set the threshold line for determining C(t) values, view and adjust an automatically generated standard curve, and interpolate samples with unknown template concentrations against the curve. If a melting analysis was performed, the melting profile, including fluorescence intensity vs. temperature and/or the negative first derivative (–dI/dt) of fluorescence intensity vs. temperature, can be viewed in the Melting Curve window.

Launching and Navigating Opticon Monitor Software

To launch the Opticon Monitor software, choose *Programs* from the Windows *Start* menu, and then *Opticon Monitor*. If you are planning to perform an experimental run, make sure the Chromo4 system is turned on, closed, and attached to the computer.

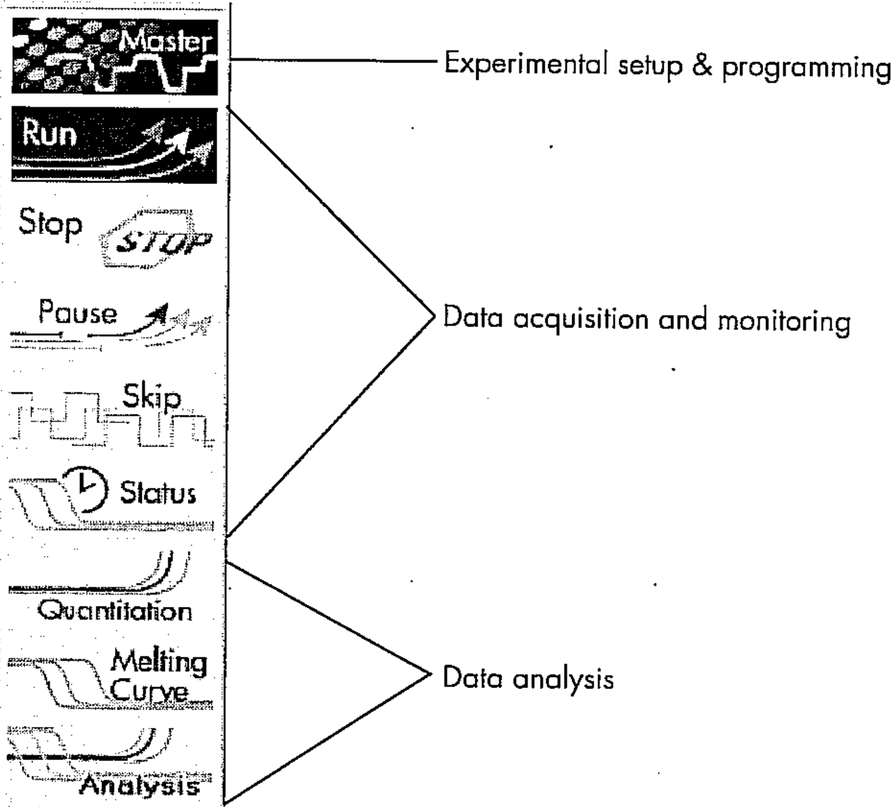
Upon first launching the Opticon Monitor software, you must accept the terms of the licensing agreement. After you click *Accept*, the Opticon Monitor window will appear displaying a new master file template. On subsequent launches, the window will display the master file template that was last used in an experimental run. Users can also launch Opticon Monitor software by double clicking on an existing Opticon Monitor master (.mast), plate (.plate), protocol (.prot), or data (.tad2) file, in which case the chosen file will be displayed.

Note: If the Chromo4 system is not attached to the computer, a message stating that the computer is unable to communicate with the Chromo4 system will appear upon launching Opticon Monitor software. The user will be instructed to make sure that the Chromo4 unit is turned on, closed, and properly connected to the computer. If Opticon Monitor software is being used for analysis only, the user may continue simply by clicking OK.

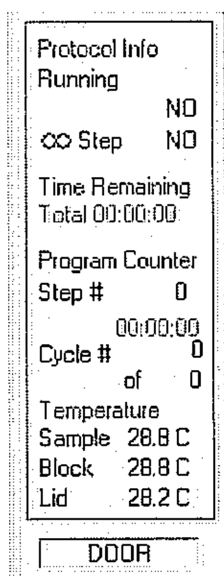


Opticon Monitor window features include:

1. **Toolbar:** The toolbar contains menu buttons that serve as the primary means of navigation between the setup/programming, run/status, and data analysis windows:



2. The **Run-Status Box** indicates whether or not a protocol or infinite incubation step is running. If a protocol is running, it lists the estimated time remaining in the run, the current step and cycle counts, and the current temperatures.



3. **Pull-down menus:** The pull-down menus provide access to many functions including the ability to export data, create calibration files for specific dyes, and view message and error logs.
4. **Setup/status/analysis display window:** This window displays the selected setup, status, or analysis screen.

Exiting Opticon Monitor Software

Exit Opticon Monitor software by selecting *Exit* from the *File* menu, or by clicking the close button in the upper-right corner of the title bar. If a protocol is running, you should stop the protocol before exiting Opticon Monitor software.

Opticon Monitor File Extensions

When saving files, the Opticon Monitor software automatically adds one of the following file extensions:

.mast **Master file:** Controls a run by specifying which plate and protocol files to apply during the run.

.plate **Plate file:** Specifies the contents of the 96 wells, any descriptive well labels that were assigned, the calibration files for the plate type and dyes used, and the amounts of any quantitation standards for use in generating a standard curve.

.prot **Protocol file:** Specifies the order and parameters of protocol steps including temperature incubations, plate reads, temperature gradients, goto steps, and melting curves.

.tad2 **Data file:** Contains the fluorescence and temperature data collected during the run, and any selected options and analysis parameters.

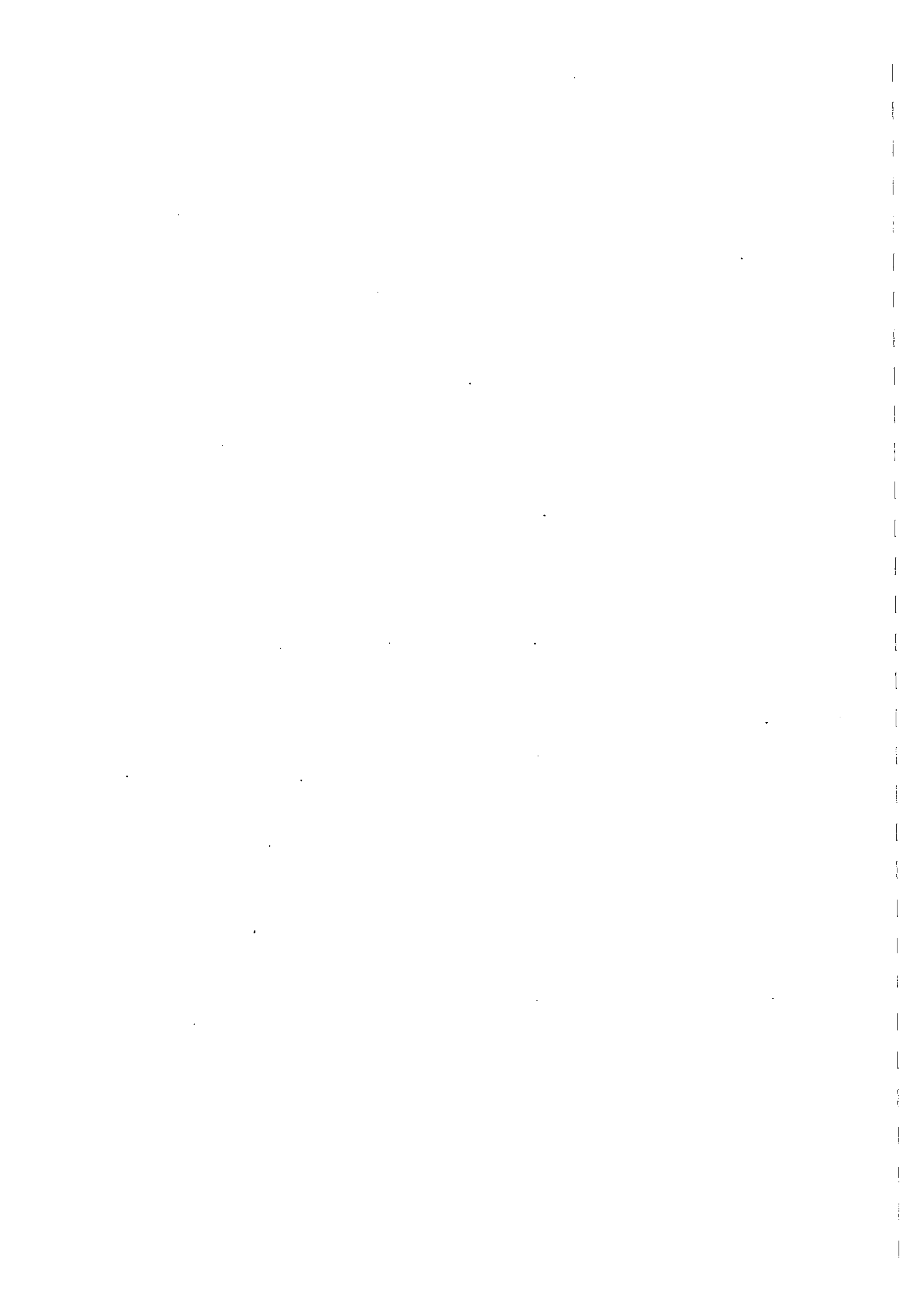
Which Version of Opticon Monitor Software Are You Running?

To determine which version of Opticon Monitor software is currently installed on your computer, choose *About Opticon Monitor* from the *Help* menu. The About window will display the Opticon Monitor version number. This manual documents Opticon Monitor software, version 2.03.

Viewing Logs

To view a record of operations performed by the Opticon Monitor software, select *Message log* from the *View* pull-down menu.

To view a list of error messages, select *Error log* from the *View* pull-down menu.



6. Experimental Setup and Programming

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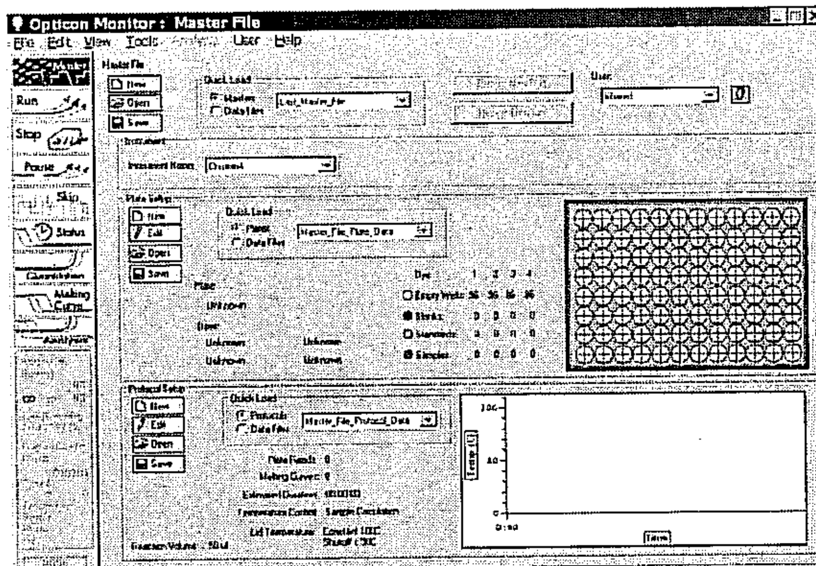
Creating a Master File

Upon launching the Opticon Monitor software (see Chapter 5), the Opticon Monitor window will appear displaying either a new master file template (first launch), or the last master file used (subsequent launches). All setup and programming operations can be accessed from the Master File window.

Before running a protocol on the Chromo4 system, the user must tell the system how the microplate is set up, and what thermal-cycling parameters to use in the run. This is done with plate and protocol files, respectively. The master file specifies which specific plate and protocol files are to be used in a run. The first section of this chapter will describe how to create new plate and protocol files and how to assign them to a master file. The second section will describe how to assign existing plate and protocol files, with or without modifications, to a master file. Finally, instructions for reusing and editing existing master files will be discussed.



New master file

A master file consists of two component files:

1. The plate file specifies the contents of the wells, any descriptive well labels that the user assigns, the calibration files for the plate type and dyes used, and the quantities of any standards to be used in generating a standard curve.

2. The protocol file specifies the order and parameters of temperature incubations, plate reads, temperature gradients, goto steps, and melting curves to be used for the experimental run. It also specifies the reaction volume, temperature control method, and heated lid settings.

The *New, Edit, Open,* and *Save* buttons located in the Plate Setup and Protocol Setup panels of the Master File window can be used to assign new or existing files to the master file as described below. Alternatively, the Quick Load menus can be used to quickly assign existing plate and protocol files to the master file (see the "Using the Quick Load Menus" section at the end of this chapter).

Instrument Selection/Re-initialization Panel

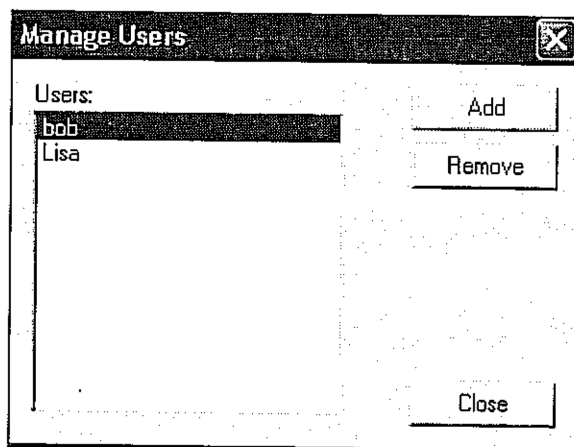
If more than one instrument is connected to the computer, use the pull-down menu in the Instrument selection/re-initialization panel to select the instrument you wish to run. This panel may also be used to re-initialize the Chromo4 detector, for example if it were not properly connected to the computer upon launching Opticon Monitor software, by selecting *Chromo4* from the pull-down menu.

Specifying a User

The User feature allows users to organize master, plate, protocol and data files by placing them in the Shared folder or into personal folders.

To select a user, click the icon in the Master File window.

In the Manage Users window that appears, highlight the name of the user you want to choose..This user's folder will become the default save location for newly saved files.



Adding New Users

To create a new user, select *Add.* Enter the new user's name in the New User window that appears and select *OK.* The new user's name will appear in the Users list

Removing Users

To remove a user from the Opticon Monitor software, select the user's name in the Manage Users window and then select *Remove.* You will be asked to confirm deletion of the user, as **all data associated with the user will also be deleted**.

Assigning New Plate and Protocol Files to a Master File

Creating a Plate File

A plate file describes the contents of the experimental microplate. The Opticon Monitor software uses the information in this file to interpret the raw data collected by the fluorescence detectors. The plate file specifies the type of microplate and dyes used in the experiment, so that the software can apply the appropriate calibration files to the data. The plate file also defines the contents (see below) of individual wells, the quantities and units of any standards, and may contain well descriptions to identify the specific reaction components.

Note to users of previous versions of Opticon Monitor software: The reaction volume is now specified in the protocol file.

One of four possible "content" types is assigned for each of the four dye channels for each well. The four content types are: "Empty" (software will ignore collected data), "Blank" (collected data may be used for background subtraction or controls), "Quantitation Standard" (software will use data for standard-curve generation), and "Sample" (the unknown and control reactions). Because contents are defined for each channel, one well may be classified as blank with regard to one dye and sample with regards to another dye.

To create a new plate file, click the *New* button in the Plate Setup panel of the Master File window. The following screen will appear. Refer back to this figure as you read the following pages of this manual, which describe the panels in more detail.

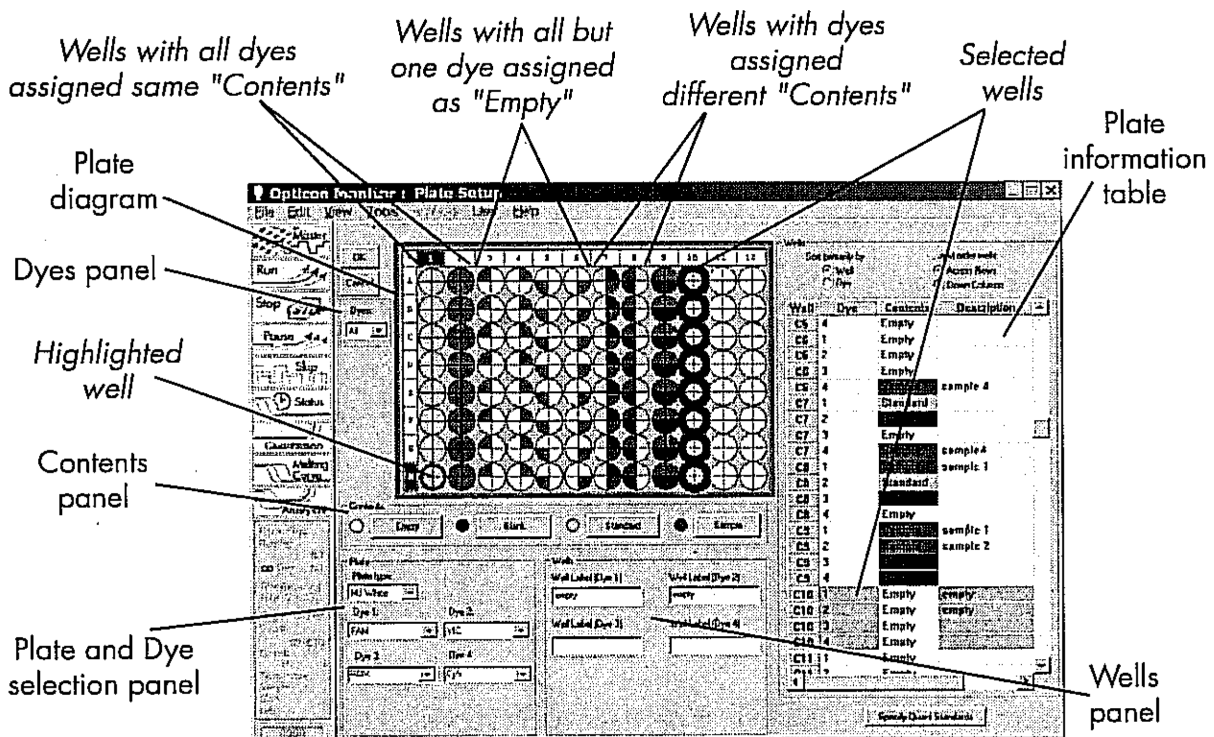


Plate file window

Parts of the Plate Setup Window

The Plate Setup window is made up of the following panels:

The Plate diagram is a representation of the 96 wells that may be used in the experiment. Each well in an experiment can be defined as containing up to four different dyes, and each dye can be used in one of three different reaction types. The wells in the Plate diagram are color-coded according to the reaction type, or "Contents", specified for each dye: white for empty (dye not used), blue for blank, green for standards, and red for sample.

The Plate diagram can display the contents for a particular dye or for all dyes. When displaying contents for all dyes, the wells in the diagram are partitioned into four quadrants. Contents for dye 1 are displayed in the upper left quadrant, for dye 2 in the upper right, for dye 3 in the lower left, and for dye 4 in the lower right.

The Dyes panel, to the left of the Plate diagram, is used to select which dye to display in the Plate diagram, or to select *All* dyes.

The Contents panel is used to indicate what type of reactions, *Empty, Blank, Standard,* or *Sample,* are present for each color dye in each well.

The Plate and Dye selection panel is used to indicate what vessel type and dyes will be used in the experiment.

The Plate information table is a list of the contents of each well, including any descriptions the user adds as a reminder of the specific contents.

The Wells panel is used to enter the descriptions that appear in the plate information table.

Selecting and Deselecting Wells

To select wells, either for assigning content for an experiment or, later, to view the data for a specific well, you can use either the Plate diagram or the plate information table to the right of the Plate diagram.

Selecting Wells Using the Plate Diagram

Move the cursor over an individual well, row letter, or column number to highlight the well or wells with a thin outline and darken the corresponding well coordinates (see well H1 in the diagram on page 6-4). Clicking on a highlighted well will select it. Selected cells appear heavily outlined, and the fields of the corresponding wells are highlighted in the plate information table (see wells in column 10 in the diagram on page 6-4).

- Select an individual well by clicking on the well.
- Select multiple wells by holding down the left mouse button and dragging the cursor over the wells to be selected, or hold down the control key and click on the individual wells you wish to select.
- Select all wells in a column by clicking on the numbered box at the top of the column. To select multiple columns, hold down the control key and click on the numbered box at the top of each column to be selected.
- Select all wells in a row by clicking on the lettered box at the start of the row. To select multiple rows, hold down the control key and click on the lettered box at the start of each row to be selected.
- Select all wells in the plate by clicking on the * in the upper-left corner of the Plate diagram.

To deselect a well(s), either click on the well(s) again or select another well or set of wells.

Selecting Wells Using the Plate Information Table

- Select an individual well by clicking on the well's coordinates (e.g., A1) in the table.
- Select multiple wells by holding down the control key and left clicking on each well's coordinates to select it.

Specifying the Plate Type and Dyes

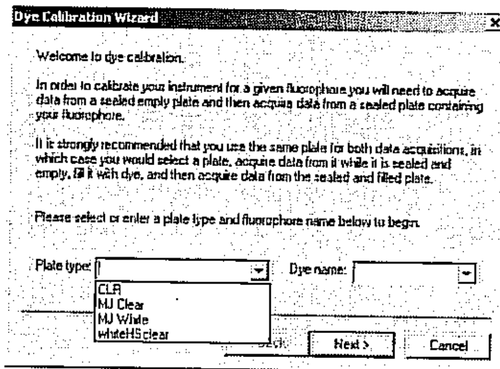
The Chromo4 detector must be calibrated for both the plate type and the dyes that will be used in an experiment. **The system comes pre-calibrated with settings for plates and many commonly used dye chemistries.** Plate calibration files exist for white or clear wells. Dye calibration files exist for Cy3, Cy5, FAM, HEX, JOE, ROX, SYBR Green I, TAMRA, TET, Texas Red, and VIC. Please note that not all dyes can be read in each channel. Dyes are only listed under the channel(s) that have the appropriate excitation and detection wavelengths for the dye.

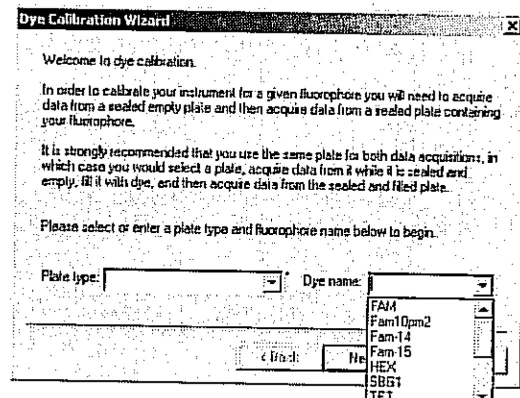
Select the desired *Plate type* from the drop-down list.

Next, select the *Dye* that should be detected in each channel. This panel tells the software which dye-calibration files to use. All channels must have a dye option applied to begin the run.

If the desired calibration file is not present for the dye/plate combination being used, it may be possible to use the setting for one of the pre-calibrated dyes, if one has excitation and emission spectra similar to those of the dye you want to use. Please contact MJ Research if you would like calibration recommendations when using non-pre-calibrated dyes.

If it is not possible to use one of the pre-calibrated settings, it will be necessary to perform a calibration by selecting *Dye Calibration* from the *Tools* pull-down menu. Follow the directions in the Dye Calibration Wizard to create a calibration file(s) for the dye(s) being used.





Specifying Dye(s) Displayed in the Plate Diagram

Use the drop down list in the Dyes panel to select which dye to display in the Plate diagram, or select *All* to display the contents for all wells at once. When *All* is selected, the wells in the Plate diagram are sectioned into quadrants. Contents for dye 1 are displayed in the upper left quadrant, for dye 2 in the upper right, for dye 3 in the lower left, and for dye 4 in the lower right.

Assigning Well Contents

If you will be performing an experiment in which all dyes will be assigned the same contents in every well, select *All* in the Dyes panel. This facilitates Plate setup by allowing you to assign the contents for all dyes at the same time.

If you will be performing an experiment in which some wells will contain different contents for the individual dyes (e.g. standard for dye 1 and sample for dye 2, or sample for dye 1 and blank for dye 2), you must assign the contents to the wells for each dye individually. First, select dye *1* in the Dyes panel. Wells in the Plate diagram will not be partitioned because only the well assignments for dye 1 will be illustrated. Follow the instructions below to assign contents to all wells containing dye 1. Then, choose dye *2* in the Dyes panel. The Plate diagram will appear empty because no contents have yet been assigned to dye 2. Follow the instructions below to assign contents to all wells containing dye 2. Repeat these steps for dye 3 and dye 4. After assigning content to wells for all dyes, to view the contents assigned to each dye simultaneously, select *All*. Lines partitioning the wells will appear, and those wells that were assigned different contents for the four dyes will display different colors in the four quadrants, accordingly.

After specifying which dyes are displayed in the Plate diagram as described above, follow the steps below to identify well contents as *Empty*, *Blank*, quantitation *Standard*, or *Sample*:

1. Select the well or group of wells to which a specific content is to be assigned, using one of the methods described in the section "Selecting and Deselecting Wells".

2. Assign the appropriate contents to selected wells by clicking on one of the four buttons in the Contents panel:

   - *Empty* (white): The well does not contain reactions for the selected dye(s). Collected data will be ignored.

   - *Blank* (blue): The well contains a blank reaction (e.g., buffer only) for the selected dye(s). Fluorescence intensity measurements from blank wells can later be used in background subtraction calculations.

- Quantitation *Standard* (green): The well contains a user-specified standard of known quantity for the selected dye(s) (see the "Specifying Quantitation Standards" section below). Fluorescence intensity readings from quantitation standards are used to plot a standard curve.
- *Sample* (red): The well contains an experimental sample (unknown or control) for the selected dye(s).

The Contents column of the plate information table lists the content assignment (empty, blank, quantitation standard, or sample) and content color (white, blue, green, or red) for all dyes for each well. The listing of wells in the plate information table can be sorted according to well or dye by selecting *Sort Primarily by Well* (or *Sort Primarily by Dye*). The wells can be further sorted by column or row. Select *Across Rows* to list the wells in order of row (e.g., A1, A2, A3); select *Down Columns* to list the wells in order of column (e.g., A1, B1, C1).

3. To change the content assignment of a well, select the well as described in step 1 and click on the desired button in the Contents panel. Make sure the correct dye is selected in the Dyes panel before changing the contents. The well's color and corresponding content information in the plate information table will reflect the content change. The content assignment for the well can be changed after the run.

Assigning Well Descriptions

To aid in sample identification, you can enter well descriptions for individual wells or groups of wells. Begin by selecting the well(s) using the Plate diagram or plate information table as described in the "Selecting and Deselecting Wells" section above. Then, type a description in the Well Label (Dye 1), Well Label (Dye 2), Well Label (Dye 3), and/or Well Label (Dye 4) field(s). The well label will be applied to the selected well or wells and appear in the Description column in the plate information table. Note that by entering the label in the Well Label panel you can label multiple wells at once. Alternatively, click on an individual row in the plate information table and type a well label directly into the well's Description field. You can also copy and paste a well label from one Description field in the table to a second Description field by first clicking on the Description field and then using control+c to copy and control+v to paste. To simultaneously paste a well label into the Description fields of multiple wells, select the wells as described above and use control+v to paste into the desired Well Label field.

Specifying Quantitation Standards

If you are using quantitation standards, click the *Specify Quant Standards* button **after** you have designated standards-containing wells. A pop-up window will appear, with a table listing all of the wells that have been designated as standards. If some wells contain standards for some, but not all dyes, then "[not a standard]" will appear in the cells corresponding to those wells/dyes (e.g. cell G1, dye #4 and cell H1, dye #4 in the figure below).

Specify Quantitation Standards

Well	Dye #1 Amount	Dye #2 Amount	Dye #3 Amount	Dye #4 Amount
A1	1	1	1	1
B1	10	10	10	10
C1	100	100	100	100
D1	1000	1000	1000	1000
E1	10000	10000	10000	10000
F1	100000	100000	100000	100000
G1	1e+006	1e+006	1e+006	[not a standard]
H1	1e+007	1e+007	1e+007	[not a standard]

Units: copies Manage

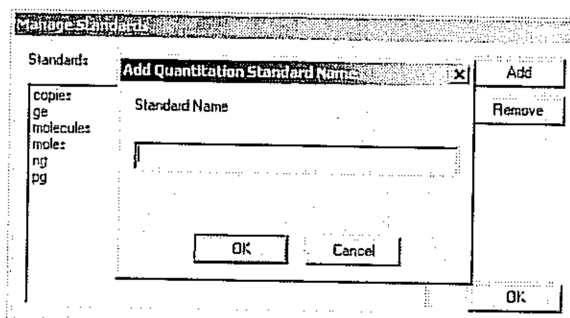
OK Cancel

To specify quantitation standards, first enter the amount of each standard in the Dye #1 Amount, Dye #2 Amount, Dye #3 Amount, and Dye #4 Amount fields as applicable. Small numbers may be entered as integers or using scientific notation (e.g., 1E4); but numbers greater than or equal to 1E7 must be written as scientific notation. Next, specify the *Units* of the standard by choosing *ng, pg, moles, molecules, ge* (genome equivalents), or *copies* from the pull-down menu. You can define additional units by selecting the *Manage* button (see below).

Select *OK* to apply the standard specifications to the plate file, or click *Cancel* to undo any changes to the standard specifications.

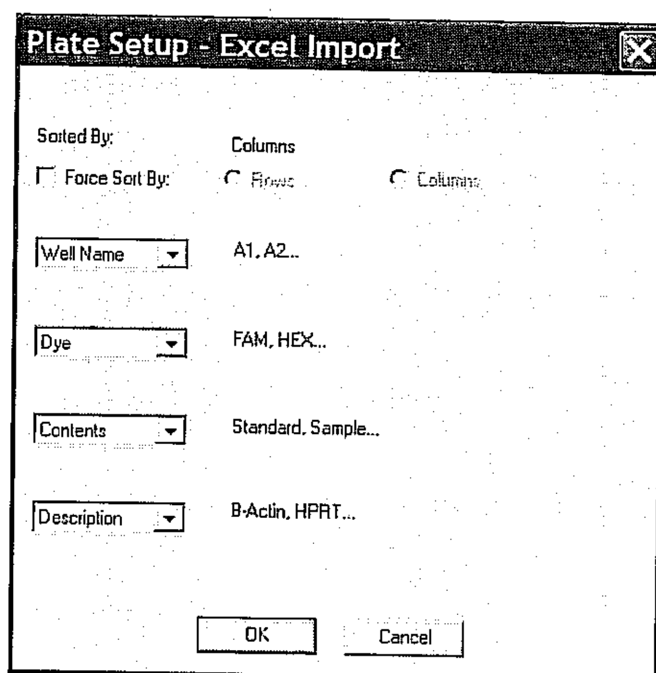
A standard curve will automatically be generated during analysis of the data using the values supplied. You will be able to adjust the standard curve by deselecting points (see Chapter 8).

To define additional units, first click on the *Manage* button in the Specify Quantitation Standards pop-up window. Next, select *Add* in the Manage Standards window that appears, and then type the desired unit name in the Add Quantitation Standard Name window. To remove unit designations, highlight the designation in the Standards list in the Manage Standards window and click the *Remove* button.



Pasting Plate Data from Excel

If on a previous run you exported a plate file to an Excel spreadsheet (see Exporting Data in Chapter 8), you may paste the plate information from the Excel file into the Opticon Monitor plate file. First, open the Excel file and select the type of plate information you want to paste (Well Name, Dye, Contents, and/or Description). Include the column headings to have these automatically applied in the Opticon Monitor software. Copy the selected cells to the clipboard. Next, return to the Opticon Monitor screen and, in the Plate Setup window, select *Paste from Excel* from the *Edit* menu. The following window will appear:



If you copied the column headings specifying the type of plate information, these headings will automatically appear in the drop-down menus in the Plate Setup – Excel Import dialog box. If the headings were not selected, or if you want to reclassify the data, select the type of plate information from the drop-down menu. Note that the data in each column must match the format for the different plate information types (Well Name, Dye, Contents, or Description). Click OK to enter the plate information into the plate information table. Note that other plate parameters, such as plate and dye type, and values of quantitation standards, are not specified by the *Paste from Excel* command.

Exiting the Plate Setup

Once you have finished entering plate file parameters, click the *OK* button in the upper-left corner of the Plate Setup window to return to the Master File window. A picture and summary of the assigned plate contents will appear in the Plate Setup panel of the Master File window.

Alternatively, if you wish to discard the plate file information and return to the Master File window, click *Cancel.*

Saving a Plate File

To save the newly created plate file, select the *Save* button from the Plate Setup panel in the Master File window. Enter an appropriate name in the File name field of the Save window. Click the *Save* button to save the .plate file.

Creating a Protocol File

The protocol file contains a program that controls the thermal-cycling parameters of an experimental run and specifies when during the run the Chromo4 detector will measure the fluorescence in the wells. Protocol steps are entered and edited in the Protocol Setup window. A listing and graphical representation of the protocol are displayed for easy review.

Click the *New* button in the Protocol Setup panel of the Master File window to create a new protocol file.

protocol creation panel

Temperature and Lid Mode panel

Reaction Volume field

Protocol List panel

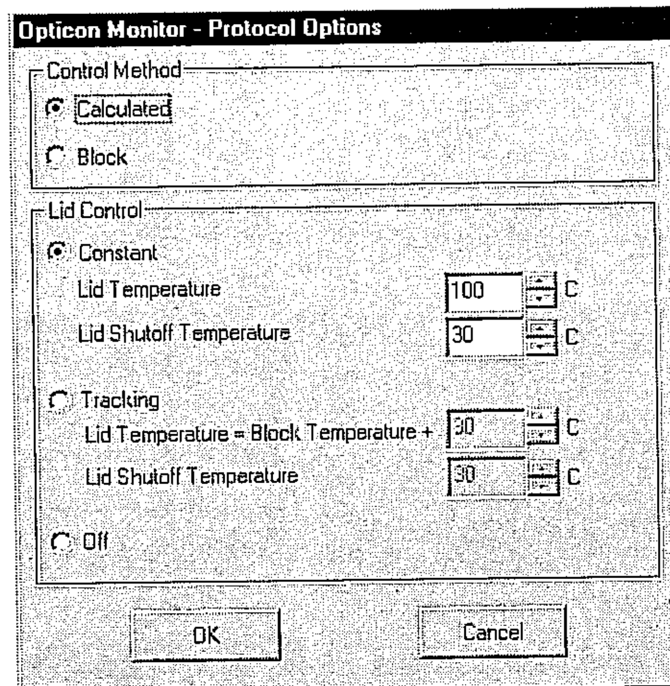
protocol editing panel

graphical representation of protocol (zoomed in)

Protocol file window

Choosing a Temperature and a Lid Control Mode

Click the *Edit* button in the Temperature and Lid Mode panel to display the Protocol Options window, and to specify the methods of temperature and lid control to be used in the run.



Temperature Control Modes

The Chromo4 system can control block temperature in two different ways. Each method has implications for the speed and accuracy of sample heating.

1. *Calculated* control is the default method of temperature control. Calculated control is the method of choice for most protocols, yielding consistent, reliable, and fast results. When using calculated control, the Chromo4 system maintains a running estimate of sample temperature based on the block's thermal profile, the rate of heat transfer through the sample tube, and the sample volume. Since this estimate is based on known quantities and the laws of thermodynamics, sample temperatures are controlled much more accurately than with block temperature control.

   Hold times can be shortened significantly when protocols are run under calculated control. Typically, for cycling denaturations running under the calculated control method, optimal hold times range from 5 to 30 seconds. Annealing/extension steps can also be shortened—but the periods for these will be reaction specific. In addition to offering the simple convenience of spending less time running reactions, shorter protocols minimize false priming and help to preserve enzyme activity.

Calculated control permits shorter protocols for three reasons:

- Brief and precise block-temperature overshoots are used to bring samples to temperature rapidly.
- Incubation periods are timed according to how long the samples, not the block, reside at the target temperature.
- The instrument automatically compensates for vessel type and reaction volume.

2. When *Block* control is selected, the Chromo4 system adjusts the block's temperature to maintain the block at programmed temperatures without calculating sample temperature. Block control provides less precision in control of actual sample temperature than calculated control provides. Under block control, the temperature of samples always lags behind the temperature of the block. The length of the time lag depends on the vessel type and sample volume but is typically between 10 and 30 seconds. Block control is used chiefly to run protocols developed for other thermal cyclers that use block control, including the PTC-100® cycler and the MiniCycler® personal cycler from MJ Research.

Reaction Volume

For the Opticon Monitor software to accurately calculate and control the sample temperature, you must enter the volume of your reactions (in µl, including the volume of any oil or wax overlay) in the Reaction Volume field in the upper right-hand corner of the protocol file window. For volumes less than 10µl enter 10µl. See the "Reaction Volume Recommendations" section in Chapter 4 for additional information.

Lid Control Modes

When a sample is heated, condensation under the tube cap or the plate cover can occur. This changes the volume of the sample, the concentration of components, and thus the kinetics of the enzymatic reaction. Use of a heated lid minimizes condensation by keeping the upper surface of the reaction vessel at a temperature slightly greater than that of the sample itself.

The Chromo4 system provides three options for controlling lid temperature: *Constant, Tracking*, or *Off.*

- The *Constant* method keeps the inner lid at a specified temperature (°C). This is the default method of control. To use constant lid-temperature control, select *Constant* and enter a Lid Temperature between 30°C and 110°C or use the arrows to scroll to the desired temperature. A temperature of 5°C to 15°C above the highest temperature in a protocol is recommended. You can also specify a sample-block temperature below which the heated lid will turn off. Enter a Lid Shutoff Temperature between 1°C and 50°C or use the arrows to scroll to the desired temperature.

- The *Tracking* method offsets the temperature of the heated inner lid a minimum specified number of degrees relative to the temperature of the sample block. Tracking is useful for protocols that have long incubations in the range of 30–70°C, in which it may be undesirable to keep the lid at a very high temperature. An offset of 5°C above block temperature is adequate for most protocols. To use tracking lid-temperature control, select *Tracking* and enter the number of degrees, from 1°C to 45°C, that you wish the lid temperature to be maintained above the block temperature, in the line *Lid Temperature = Block Temperature +* __°C. You can also use the arrows to scroll to the desired temperature. To specify a sample-block temperature below which the heated lid will turn off, enter a Lid Shutoff Temperature between 1°C and 50°C or use the arrows to scroll to the desired temperature.

Note: Because there is no active cooling of the lid, a decrease in the lid temperature may not be observed during rapid cycling. Note also that the lid heats more slowly than the sample block due to its additional thermal mass.

- When *Off* is selected in the lid control panel, no power is applied to the heated lid. In this mode, condensation will occur at a rate consistent with the incubation temperature and the type of tube or plate sealant being used. This option is recommended only when using an oil or wax overlay.

Click the *OK* button to apply the temperature and lid control settings to the protocol, or choose *Cancel* to close the window without changing the settings applied to the protocol. The Temperature Control and Lid Settings will appear in the appropriate fields of the Temperature and Lid Mode panel in the Protocol Setup window.

Designing and Entering a Protocol

Programming the Chromo4 system consists of entering a series of steps comprising a protocol. This section will present a sample protocol and describe how to enter the protocol steps. Additional protocol options will also be described.

Consider the following example protocol:

1. Incubate at 94°C for 1 minute
2. Optimize annealing temperature by incubating at a range of 55°C to 65°C across the 12 columns of the sample block, for 30 seconds
3. Read the fluorescence intensity of the Blank, Quantitation Standard, and Sample wells
4. Incubate at 72°C for 1 minute
5. Sequentially repeat steps 1–4, 29 more times, then proceed to step 6
6. Characterize reaction products by melting profile: Raise the temperature from 55°C to 90°C, and read the fluorescence 10 seconds after every 1°C increase in temperature

Entering a New Protocol

As you insert steps into a protocol, descriptions of the steps will appear in the Protocol List panel of the Protocol Setup window, and a graphical representation of each step's temperature and time period (in minutes) will appear in the Protocol Graph panel. To view the graphical display more clearly, use the sliders to the left of the graph panel to zoom in and out on the X and Y axes. Use the scroll bars on the axes to position the graph appropriately. An example of a zoomed protocol, showing only the first five steps, is shown on page 6-13.

Before beginning to enter a new protocol, note that the END step is highlighted in the Protocol List panel. Opticon Monitor software adds new steps immediately before the step that is highlighted.

Temperature Step

A temperature step specifies incubation temperature and duration. The Chromo4 system ramps the sample to this temperature at its maximum rate unless ramp modifying instructions are added to the program (see the "Manual Ramp Rate" description near the end of this section).

Click the *Temperature* button to enter a temperature incubation step (e.g., step 1 or step 4 from our example) into a protocol.

Select Step to Insert Before Line
Temperature
Set temperature to: 30.0 C
Maintain temperature for: 00:00:00
Forever
Options
Manual ramp rate 2.0 C / second
Change temperature 5.0 C / cycle
Change time 10 seconds / cycle
Beep when completed
Insert
Cancel

Enter the desired temperature, from 0°C to 105°C, into the *Set temperature to* field or use the arrows to scroll to the desired temperature—which is 94°C in step 1 of our example.

Enter the desired incubation time, to a maximum of 18 hours, in the *Maintain for* field. Click in the hour: minute: or second field and either enter a time period or use the arrows to scroll to the desired time—00:01:00 in step 1 of our example. Alternatively, you can select *Forever* to maintain the desired temperature for an infinite period of time. A forever incubation step at the end of a protocol can be useful for holding reaction products at a sub-ambient temperature (we recommend 10°C) until they can be processed. A forever incubation can also be useful as the first step in a program, for example, to bring the block and lid to the denaturation temperature before the reaction vessel is put into the block. In both cases, the SKIP button is then used to proceed to the next step of the protocol (See Chapter 7).

In the graphical representation of the protocol, a forever incubation is indicated with the symbol.

Click the *Insert* button to add the temperature step to the protocol without further modifications. The temperature step should appear as step 1 in the upper protocol display window, and a graphical representation of the temperature and duration should appear in the lower window. Note that the END step is again highlighted indicating that the next step will be added immediately above the END step and therefore after step 1.

You can also choose to modify a temperature step before inserting it into the protocol by adding options. Available options include:

1. *Manual ramp rate*: Set a slower-than-maximum rate of heating or cooling. A slower-than-maximum ramp rate ranging from 0.1°C to 2.5°C per second can be specified. Fast thermal ramping between incubation steps can often help reduce overall reaction times by 10% to 30% and may help reduce production of non-specific products.

2. *Change temperature*: Modifies a temperature step to allow a "per cycle" increase or decrease of temperature (0.1°C to 10.0°C per cycle) each time the step is executed. This feature is useful when annealing stringency is a consideration, such as in a touchdown program.

   In a touchdown program, the temperature selected for the annealing step begins higher than the calculated annealing temperature and incrementally decreases with each cycle, first reaching, and eventually falling below, the calculated annealing temperature. Thus the reaction begins at a temperature favoring high stringency in hybridization and increments to a lower stringency. The higher initial stringency favors the desired product by creating a high signal-to-noise ratio in the early amplification cycles.

3. *Change time*: Modifies a temperature step to allow a "per cycle" lengthening or shortening of the hold time of the step (by 1–60 sec/cycle) each time the step is executed.

   The change-time capability is useful for slowly increasing (typically by 2 to 5 seconds per cycle) the hold time during an extension step. The number of bases that a polymerase must incorporate during the extension step increases in later cycles because there are more template molecules and/or fewer active polymerase molecules. Allotting extra time for extension can allow synthesis to be completed.

4. *Beep when completed*: Modifies a temperature step so the instrument will beep when the target temperature is reached.

Gradient Step

The temperature gradient feature allows you to optimize denaturing or annealing conditions by incubating at several different temperatures simultaneously. The range of temperatures that can be achieved from left to right across the 96-well sample block can be as small as 1.0°C or as great as 24.0°C. The maximum programmable temperature is 105.0°C; the minimum programmable temperature is 30.0°C.

Click the *Gradient* button to insert a gradient step into a protocol.

Select Step to Insert Before Line
Gradient
Left Temperature (Lower) 55
Right Temperature (Higher) 65
Maintain temperature for: 00:00:30
Forever
Options
Change Time 10 seconds / cycle
Insert Cancel

The minimum gradient temperature is assigned to the far left column (column #1) of the sample block and can range from 30°C to 104°C. In our example, the Left Temperature (Lower) field is set to 55. The maximum gradient temperature is assigned to the far right column (column #12) and can range from 31°C to 105°C. In our example, the Right Temperature (Higher) field is set to 65. The minimum temperature differential between the far left and far right columns is 1.0°C and the maximum differential is 24.0°C. A warning will appear if you attempt to enter a differential that exceeds 24.0°C.

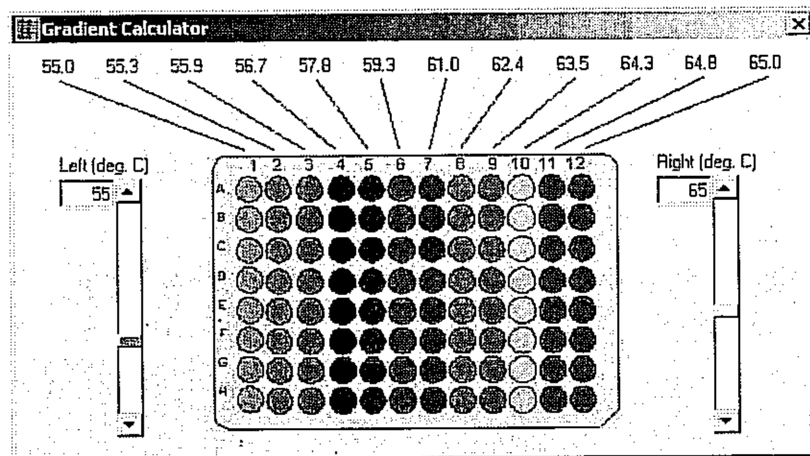
After entering the range of temperatures for the gradient, enter the desired incubation time in the *Maintain Temperature for* field by clicking in the hour: minute: or second field and either entering a time period or using the arrows to scroll to the desired time — 00:00:30 in step 2 of our example. Alternatively, you can select *Forever* to maintain the desired temperature gradient for an infinite period of time.

Click *Insert* to add the gradient step to the protocol without further modifications. The gradient step should appear as step 2 in the protocol display window. Note that the END step is again highlighted indicating that the next step will be added above the END step and therefore after step 2.

You can also choose to modify the gradient step before inserting it into the protocol by choosing the *Change Time* option (see the "Temperature Step" section for an explanation of this option).

Gradient Calculator

To accurately predict the temperature of each of the twelve columns across the block during a gradient incubation, select *Gradient Calculator* from the *Tools* menu.



Note that the gradient temperature distribution is not linear but, rather, has a broader spread in temperature between the center columns of wells. This is a natural consequence of the geometry of the Peltier-Joule heaters that underlie the block. Although not evenly distributed, the temperatures displayed are quite accurate for each well in a given column (±0.4°C of actual column temperature). The indicated temperature of the column that yields the best reaction results can be accurately transferred to a temperature step in a non-gradient protocol.

Plate Read Step

Insertion of a plate read step directs the Chromo4 detector to measure the fluorescence of the wells. The plate read begins immediately after the programmed end of the previous incubation step, step 2 in our example. The Chromo4 detector performs the plate read at the current incubation temperature, and then initiates the next step, step 4 in our example.

To insert a plate read step, click the *Plate Read* button. A plate read step will be inserted into the protocol. In our example, the plate read step should appear as step 3 in the Protocol List panel. A plate read appears as an eye icon in the graphical protocol display.

Adding Multiple Temperature Steps, Gradient Steps, or Plate Reads

To add additional temperature steps, gradient steps, or plate reads to your protocol, click the appropriate button and follow the directions for the specific step as outlined above.

Following our example, step 4 is a temperature incubation step of 72°C for a duration of 00:01:00 with no additional options.

Goto Step

The goto step abbreviates a protocol that has many repeating steps. When a goto step is executed, the protocol program returns to the step specified by the goto step, and repeats that step and all subsequent steps back to the goto step. After it has looped back to the goto step a specified number of times, the protocol program moves on to the step that follows the goto step. **Note:** You cannot nest goto steps. In other words, a protocol loop cannot contain another goto step within this loop. You cannot include melting curve steps (see next section) within a goto loop.

Step 5 of our example protocol indicates that the protocol should return to step 1, repeat steps 1–4 an additional 29 times for a total of 30 cycles, and then proceed to step 6. To add the goto step, click the *Goto* button in the protocol creation panel.

Enter the line number of the step to which the protocol program should return. You may also select the number of the step using the drop-down menu, which is accessed by clicking the arrow to the right of the *Go to line* field. In our example, enter 1 in the *Go to line* field, and 29 in the *How many more times?* field. Step 5 of our protocol will then direct the Chromo4 detector to repeat steps 1,2,3, and 4 of the protocol 29 times before continuing on to step 6. (Steps 1–4 will be performed a total of 30 times.)

Click the *Insert* button to add the goto step to the protocol.

Melting Curve Step

A melting curve can be performed to identify specific products and/or assess the homogeneity of a sample. Melting curve profiles are influenced by several factors, including the number and concentration of discreet fragments produced, the length and G+C content of each fragment, and buffer conditions.

Melting curves are often useful in verifying the identity of amplification products, as well as distinguishing positive internal controls from amplified products. In these cases, simply specify a melting curve after a cycling run, and the instrument will perform both procedures automatically.

The Chromo4 system can also be programmed to run a melting curve independent of a cycling protocol. This analysis can be useful in a variety of applications including homothermic assays, sizing fragments relative to ladders, and utilizing the 96-well capacity of the Chromo4 detector to perform endpoint assays to increase throughput.

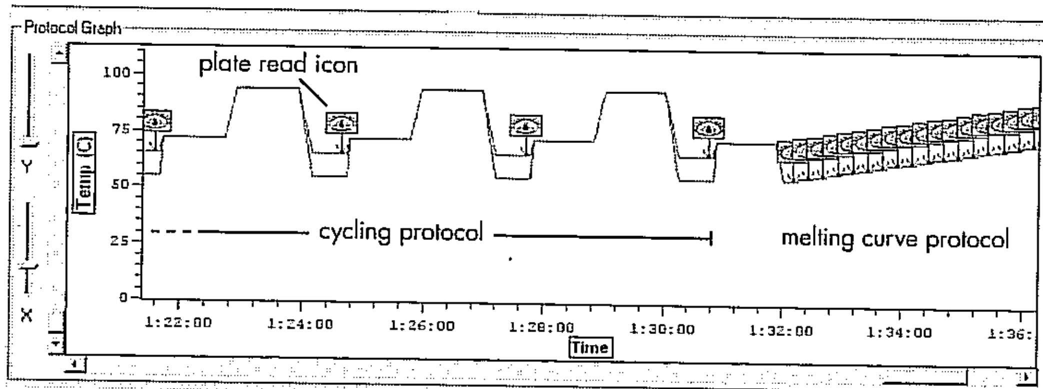
To add a melting curve step, click the *Melting Curve* button in the protocol creation panel.

Select Step to Insert Before Line
Melting Curve
Start temperature: 55.0 C
End temperature: 90.0 C
Increment: 1.0 C
Hold time: 00:00:01 + Plate Read
Insert Cancel

Enter a *Start temperature* (0.0°C to 99.0°C), and an *End temperature* (1.0°C to 100.0°C). In our example, the melting curve starts at 55°C and ends at 90°C.

Next, specify when during the melting curve step the Chromo4 detector will measure fluorescence. Designate a temperature *Increment* between reads of 0.1°C to 10°C and a *Hold Time* between reads (1 second to 1 hour) corresponding to the duration for which the temperature increment should be maintained before the fluorescence is read. A temperature increment of 0.2°C and a hold time of 5sec is recommended for many protocols.

Click the *Insert* button to add a melting curve step to the protocol. Data can be analyzed from only one melting curve step per protocol.



Editing a Protocol Step

To edit a protocol step, first click on the step in the protocol display window to highlight it. Then, click the *Edit Step* button in the protocol editing panel. The parameters for the step as it is currently entered will appear. After making the desired changes, click the *Replace* button to enter the edited step into the protocol, or click *Cancel* to leave the step unmodified.

Deleting a Protocol Step

To delete a protocol step, click on the step in the protocol display window to highlight it . Then, click the *Delete Step* button in the protocol editing panel to remove the step from the protocol. The remaining protocol steps will automatically renumber.

Inserting a Protocol Step Between Existing Steps

To insert a protocol step between existing steps, highlight the step in the protocol display window that will follow immediately after the newly inserted step. All protocol steps are added immediately before the step that is highlighted in the protocol display window. Next, click the button corresponding to the type of step you would like to add. After you click *Insert*, the new step will be added and the protocol will be renumbered.

Exiting the Protocol Setup

Once you have finished entering protocol-file parameters, click the *OK* button in the upper-left corner of the Protocol Setup window to return to the Master File window. A graphical representation of the protocol and a summary of the total number of plate reads, melting curves, and the estimated run duration will appear in the Protocol Setup panel of the master file window.

If you wish to discard the protocol file information and return to the master file, click *Cancel*.

Saving a Protocol File

To save the newly created protocol file, select the *Save* button from the Protocol Setup panel in the Master File window. Enter an appropriate name in the File name field of the Save window. Click the *Save* button to save the .prot file.

Saving a Master File

To save a master file, which will contain the plate and protocol files displayed in the Master File window, click the *Save* button in the Master File panel. Enter an appropriate name in the File name field of the Save window. Click the *Save* button to save the .mast file.

You can also choose to not save this collection of component files and proceed directly to the run (see Chapter 7 for information on initiating a run).

Assigning Existing Plate and Protocol Files to a Master File

To assign existing plate and protocol files to a new or existing master file, either click the *Open* button in the panel of the master file window corresponding to the type of file you wish to assign, or use the Quick Load menus to assign existing plate/protocol files to a master file (see the "Using the Quick Load Menus" section below).

Selecting *Open* will display all the plate/protocol files in either the Shared folder or, if a user has been assigned to the master file, in that user's folder. Select the desired file or, if the desired file has been saved to an alternate location, use the Windows browse screen to locate the file, and then click *Open*. The plate/protocol file will be applied to the master file, and a corresponding summary will appear in the Master File window.

To view the newly assigned plate or protocol file and/or make any necessary modifications, click the *Edit* button in the appropriate panel of the Master File window. The Plate Setup or Protocol Setup window will open allowing you to modify the file parameters. Select *OK* to retain any modifications and return to the Master File window, or select *Cancel* to return to the Master File window without modifying the plate/protocol file. Select *Save* to save any changes to the plate/protocol file under the same or a newly assigned file name. See the "Saving a Plate/Protocol File" sections in this chapter for additional information on saving plate/protocol files.

Click the *Save* button in the Master File panel to save any changes to the master file.

Reusing Master Files

A new master file need not be created for every run. Existing master files may be reused without modifying the plate or protocol files, or the master file may be edited to accommodate changes such as a different arrangement of samples in the plate.

If a run has just completed, or if a data file for a previously completed run has just been displayed, and you wish to use the same master file, click on the *Repeat This Run* button in the Master File window. To access a new master file template, click on the *Prepare New Run* button. To display an existing master file, click the *Open* button in the Master File panel, or use the Quick Load menu to assign an existing master file to the master file template (see the "Using the Quick Load Menus" section below). To access a recently used master file, select the desired master file from the list of *Recent Master Files* accessible from the *File* menu.

Selecting *Open* will display all the master files in the Shared folder or, if a user has been assigned to the master file template, in that user's folder. Select the desired file or use the Windows browse screen to locate the file if it has been saved to a different location, and then click *Open*. The master file will be applied to the master file template and the corresponding plate and protocol file summaries will appear in the Master File window.

To use the master file without any changes, proceed to Chapter 7, *Run Initiation and Status*.

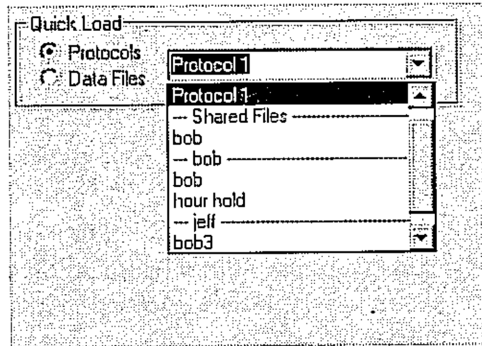
You can also modify the master file before initiating a run, by editing the assigned plate or protocol files (*Edit* button), by substituting files (*Open* button or *Quick Load*), or by creating new component files (*New* button).

Important Note: If a component plate or protocol file is edited and saved under the same name, the edited file will replace the original file in all master files to which that file has been assigned.

Click the *Save* button to save any changes to the master file.

Using the Quick Load Menus

The Quick Load menus provide rapid access to any existing plate, protocol, master, or data files. If the *Plates/Protocols/Masters* option is selected, all of the available plate, protocol, or master files that have been saved to the Shared folder or individual user folders are displayed in the drop-down menu. The files are listed along with their associated user as shown below.



Scroll to locate the desired file in the drop-down menu, and select the file. If the *Data Files* option is selected, all of the data files are listed in the drop-down menu. Selecting a data file will apply the plate, protocol, or master file that was used to generate that data file to the current master file template.

7. Run Initiation and Status

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Monitoring Run Status, 7-3
Protocol Information in the Run-Status Box, 7-3
The Status Window, 7-4
Thermal Cycler Status, 7-4
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Performing Data Analysis during a Run, 7-6

Running a Protocol

Before initiating a run, check that the appropriate master file is displayed. Click the *Master* button on the toolbar to access the Master File window if it is not currently displayed. If a run has just completed or a data file for a previously completed run has just been displayed, select the *Prepare New Run* button to access a new master file template, or select the *Repeat This Run* button to use the same master file (see Chapter 6 for instructions on how to create and edit master files).

Initiate the run by clicking the *Run* button on the toolbar. A Windows browse screen will appear asking you to name the file to which the data will be saved. Click *Save* to accept the default filename, or enter an appropriate file name and then click the *Save* button. The data file will be saved as a .tad2 (acquired data) file. The file will be saved in the data folder of the selected user (see *Specifying a User*, in Chapter 6).

The default data filename can be changed using the *Filename Preferences* command under the *User* menu. The fields that can be included in the default filename are: username, year (two format choices), month (two format choices), day (two format choices), time (six format choices), and count, which adds an incremental count to the filename. In addition to these variable fields, the default filename can include constant characters, for example an underscore between fields. The fields and constant characters must be typed into the Data Filename Format field in the Filename Format window, as in the following example:

<year1>_<month2><day1>_<time4>

This setting will result in a default filename as follows:

2003_March15_102013PM

Interrupting a Run

Click the *Stop* button on the toolbar to halt the run at any time. The *Skip* button can be used to skip to the next step in the protocol file. **Note that if you press *Skip* while a Goto loop is being executed, the program will skip out of the loop and implement the next protocol step. Pressing *Skip* does not skip to the next step within the loop.** For example, if step 8 of a protocol says to goto step 2 for 39 times, and you press *Skip* during the 20th execution of step 5, the program will skip to step 9 of the protocol, not step 6.

Monitoring Run Status

Protocol Information in the Run-Status Box

A summary of run information is displayed in the status box located below the toolbar. The run summary includes:

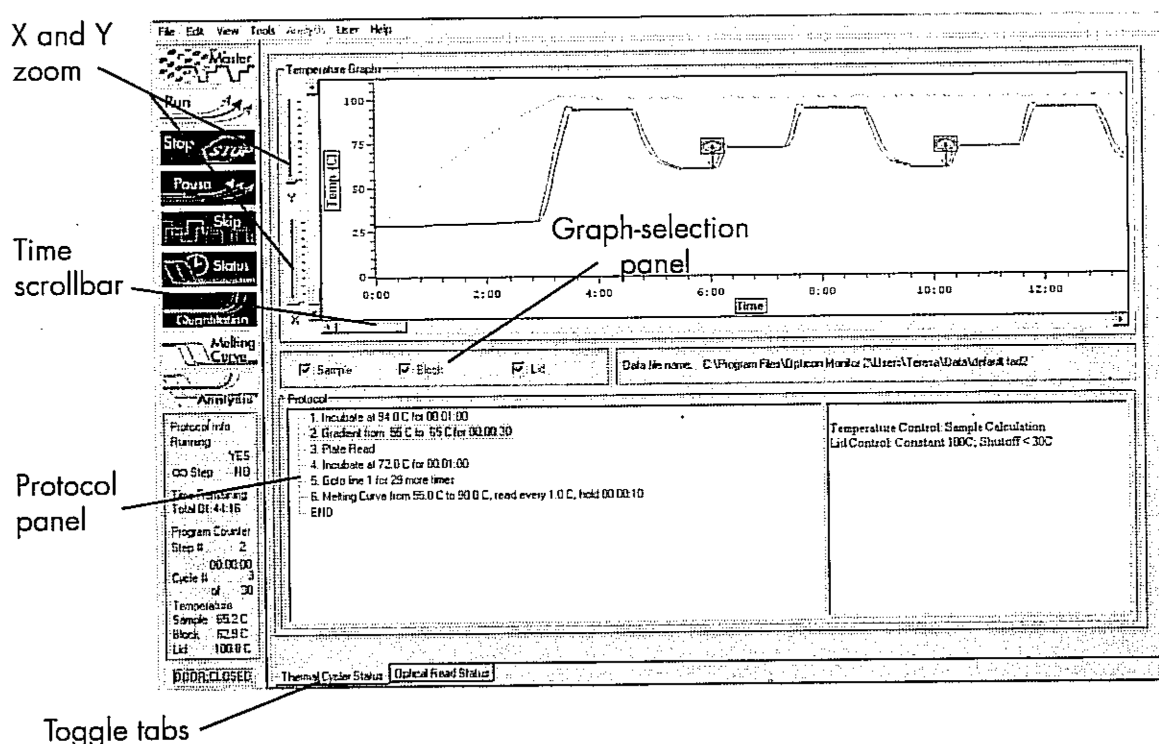
- Protocol information: indicates whether a protocol or a forever incubation is currently running.
- Time Remaining: displays an estimate of the time remaining for the run.
- Program Counter: displays the current step number, current cycle number, and the total number of cycles in the protocol.
- Temperature: displays the current sample, block, and lid temperatures.

Protocol Info
Running
YES
∞ Step NO
Time Remaining
Total 01:44:59
Program Counter
Step # 1
00:00:49
Cycle # 3
of 30
Temperature
Sample 94.0 C
Block 94.0 C
Lid 101.0 C
DOOR:CLOSED

The Status Window

The Status windows allow you either to monitor run progress, by viewing the Thermal Cycler Status screen, or to monitor real-time data collection, by viewing the Optical Read Status screen. The Thermal Cycler Status screen is automatically displayed in the Status window when the run is initiated. To access the Status window after the run has completed, click the *Status* button on the toolbar. The tabs in the lower-left corner of the Status window toggle the display between the Thermal Cycler Status and Optical Read Status screens.

Thermal Cycler Status Screen

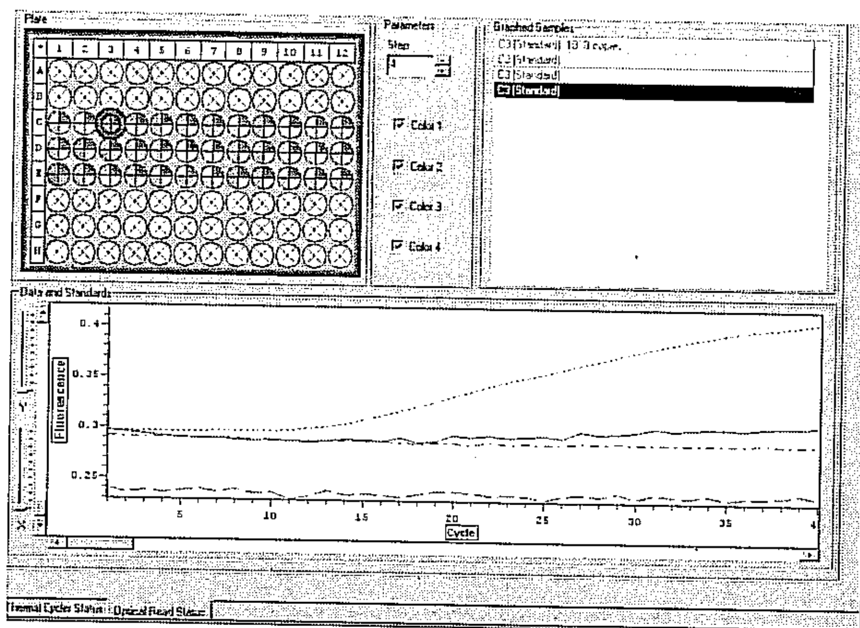


Thermal-cycler status window

The run status is graphically displayed in the top portion of the Thermal Cycler Status window. Select the appropriate boxes in the graph-selection panel to display a graph of the *Sample*, *Block*, and/or *Lid* temperatures over time. To more clearly view a small section of the graph(s), use the X and Y zoom sliders to the left of the graph, or right click and drag a box around the area of the graph that you wish to magnify. The protocol is listed in the bottom portion of the window with the current step highlighted. The END step is highlighted if the run has finished.

Optical Read Status

Click the *Optical Read Status* tab to monitor real-time data collection.



Optical read status window

A graph of fluorescence versus cycle number can be displayed for the selected wells. Use the Plate diagram to select the wells to be included in the graph (see the "Selecting Wells Using the Plate Diagram" section in Chapter 6 for additional information). Note that no data will appear in the graph until you select a well. Select *Color 1* to display the graph for dye 1, *Color 2* to display the graph for dye 2, etc. Each dye will be plotted with a different line style.

Selected wells will appear outlined in color. The color outlining the well corresponds to the color of the well coordinates in the Graphed Samples list and to the color of the fluorescence intensity trace in the graph.

Deselect all wells by clicking on any selected well. To deselect a subset of wells, hold down the control key, and click on the well(s) you wish to deselect. The well(s) will no longer appear outlined in color, and the corresponding fluorescence intensity trace will be removed from the graph.

To highlight the results for a particular sample in the Plate diagram, Data and Standards graph, and the Graphed Samples list:

- Move the cursor over a well on the Plate diagram. The column number and row letter coordinates of the well will be highlighted in the Plate diagram. The well will also be highlighted in the Graphed Samples list, and its trace will be thickened on the Data and Standards graph.
- Move the cursor over a particular trace on the graph to thicken the trace and display the x and y coordinates corresponding to the position of the cursor on the trace. The corresponding well in the Plate diagram and the well coordinates in the Graphed Samples list will also be highlighted
- Select a well from the Graphed Samples list to highlight the well coordinates in the Plate diagram and thicken its trace on the Data and Standards graph.

Use the Step field in the Parameters panel to display the signal intensity data for a particular step, if a plate read is included in more than one step of the protocol. This can be particularly useful if, for example, you wish to monitor a plate read in step 3 and later a melting curve in step 6.

Performing Data Analysis During a Run

The Opticon Monitor software allows users to analyze data while a protocol is running. Quantitation, Analysis, and Melting Curve screens can be used to view the data collected so far in the current run (see Chapter 8 for more details about these screens). It is also possible to analyze data collected during a previous run while a current run is in progress. To analyze previously gathered data during a run, select the *File->Open->Data File* path you would normally use to open a data file. The Status, Quantitation, Melting Curve and Analysis windows will display the data from the previous run and data analysis can be performed as usual in these windows. The Protocol Info in the run-status box (in the lower-left corner of the screen) will continue to display the status of the current run. In order to close the data set and return to the current protocol running, select the *Reattach to Run* option located under the *File* menu.

It is also possible to open a second Opticon Monitor session while a run is in progress—simply follow the same steps used to open the initial session.

8. Data Analysis

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Basic Principles of Quantitation

Continuous real-time fluorescence detection of amplification products allows accurate calculation of the initial quantity of template present in a sample. This is possible because there is a highly reproducible relationship between the initial amount of template present and the number of cycles required before a significant increase in fluorescence signal is observed during the exponential phase of amplification. The larger the initial template number, the fewer cycles required before significant fluorescence signal is detected. Based on this relationship, the number of cycles completed before signal is detected can be used to calculate the initial quantity of template in a sample.

To calculate the initial quantity of template in a sample, one must first define the threshold above which fluorescence signal is deemed significant, using a graph of fluorescence vs. cycle number. The threshold line is positioned on a graph of baseline-subtracted data at a point where the signals surpass background levels and begin to increase exponentially. The threshold cycle, C(t), for an individual sample is then defined as the cycle at which the sample's fluorescence trace crosses the threshold line.

By including quantitation standards with a range of known initial amounts of template in the run, one can generate a linear standard curve of the log of the template quantity vs. C(t) value. The quantity of initial template in unknown samples can then be calculated by interpolating the sample's threshold cycle against the standard curve. On initial runs, or for applications requiring a high degree of precision, including replicate quantitation standards in the run can aid in positioning the threshold line. The use of replicates allows one to determine which options for setting the threshold line parameters—described later in this chapter—provide the tightest fit of the replicates onto the standard curve.

Quantitation in Opticon Monitor™ Software

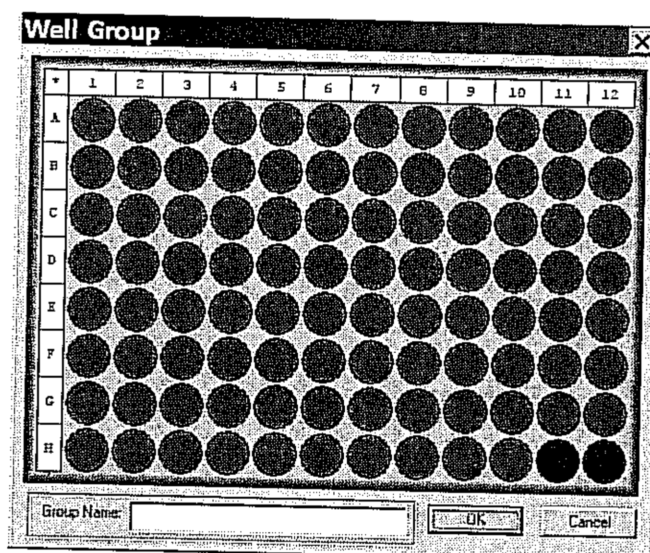
Use the Quantitation window to analyze Data and Standards graphs, adjust the data analysis options, position the threshold line, adjust the automatically generated standard curve, and calculate the quantity of sample initially present in a reaction. If a run is in progress or has just completed, click the *Quantitation* button on the toolbar to analyze the quantitation data.

To access a previously generated data file, select *Open* and then *Data File* from the *File* menu. Select the desired file from the standard browsing window. To view a recently created data file, select the desired file from the *Recent Data Files* list accessible from the *File* menu. When the data file loads, the Quantitation window is displayed.

Managing Well Groups

In some cases, you may want to analyze only a subset of the data collected during a run, for example if two separate experiments were run at the same time. The Manage Well Groups function allows you to define subsets of wells to be independently analyzed. This is especially useful if two experiments with different quantitation standards were run simultaneously. By default, Opticon Monitor software uses all wells classified as standards to generate a standard curve, as described in the next section. If the Manage Well Groups function is used to define subsets of wells, then when one of those groups is selected, the Standards graph will include only the standards present in that particular group.

To define well groups, select *Manage Well Groups* from the *Quantitation* menu. A window listing any defined groups will appear. To create a new group, click the *Add New...* button. A new window will open, with a plate diagram from which you can select the wells to include in this group (see "Selecting Wells Using the Plate Diagram" in Chapter 6).

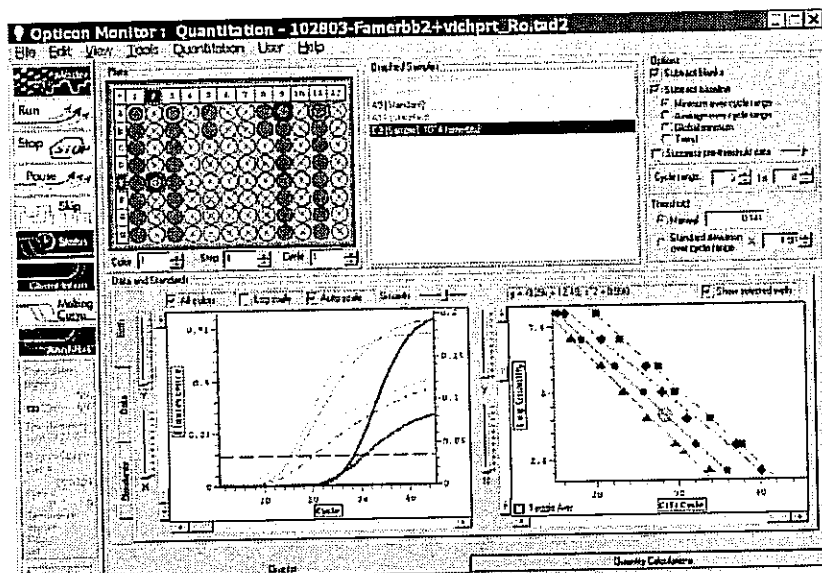


Once you have selected all wells to include, enter a name in the Group Name field and click *OK*. The newly defined group now appears in the Well Groups list. You may now define additional groups by clicking *Add New...*, edit an existing group by highlighting it and clicking *Edit...*, delete a group by highlighting it and clicking *Delete*, or perform quantitation and analysis on a group by highlighting it and clicking *Activate*. When you click on *Activate*, the Well Groups window automatically closes, and all the wells that are not in the activated group appear as empty in the Plate diagram (i.e. they are white with an X through them). To activate a different group, you may either re-select *Manage Wells* from the *Quantitation* menu and proceed as above, or simply select a defined group from the *Select Well Group* submenu of the *Quantitation* menu.

When a well group is activated, its name appears in the title bar at the top of the Opticon Monitor window.

Graphs

The Graphs screen is the default Quantitation screen.

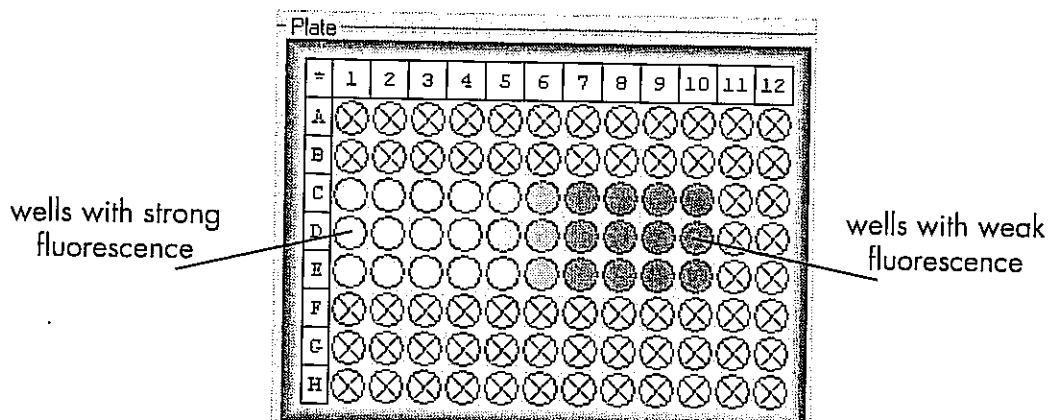


The Quantitation Graphs Screen

A *Data* graph of fluorescence (or log fluorescence) versus cycle number, a *Standards* graph of log quantity versus C(t) Cycle, or *Both* can be displayed by clicking on the appropriate tab on the left side of the bottom panel of the Quantitation window.

Using the Plate Diagram to View Fluorescence Intensity

The Plate diagram can be useful for selecting wells to include in the Data graph and for determining approximate fluorescence intensity that was measured in the wells during each cycle. The interior color of a well in the Plate diagram correlates with the signal intensity measured in that well for the specific Color, Step, and Cycle specified in the fields below the diagram. Dark grey or black wells indicate weak or no signal, whereas white or light grey wells indicate strong signal. Normally, if you use the arrows adjacent to the Cycle field to scroll through the cycles, the wells will get lighter as the cycle number increases, indicating that the fluorescence intensity is increasing. By scrolling to the last cycle, you can get an indication of the endpoint fluorescence intensity in each well.

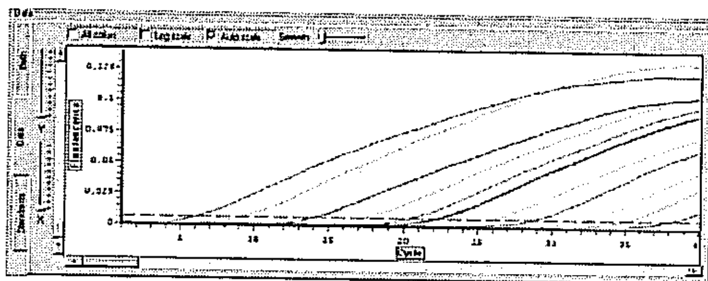


Use the Color field to view the fluorescence intensity for dye *1*, *2*, *3*, or *4* in both the Plate diagram and the Data graph (see the "Data Graph" section immediately below). To display the fluorescence data for a particular step, if a plate read was performed at more than one step of the protocol, enter the step number in the Step field or use the arrows to scroll to the desired step. Use the Cycle field to view (in the Plate diagram) the signal intensity measured during a specific cycle. You can also use the Cycle field to mark a particular cycle in the Data graph with a vertical dotted line.

Data Graph

Use the Plate diagram to select the wells to be included in the Data graph. See the "Selecting Wells Using the Plate Diagram" section in Chapter 6 for additional information. Note that no graph will be displayed until a well is selected.

Click the *Data* tab on the left side of the graph panel to display a large graph of fluorescence vs. cycle number.



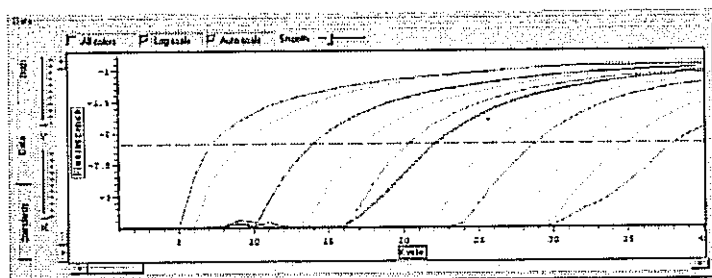
Selected wells will appear outlined in color in the Plate diagram. The color that outlines a well is the same as that used to color that well's coordinates in the Graphed Samples list, and to color that well's fluorescence intensity trace in the Data graph. The fluorescence intensity data for the color that is selected in the Color field (below the Plate diagram) will be displayed in the Data graph. If the *All colors* option is selected in the box at the top of

the Data graph panel, the intensity data for the dye selected in the Color field will be drawn with a solid line, and the data for the other dyes will be drawn as differently styled (dash, dot-dash, dot-dot-dash) lines of the same color.

Deselect all wells by clicking on any selected or empty well. To deselect a subset of wells, hold down the control key, and click on the well(s) you wish to deselect. The well(s) will no longer appear outlined in color, and the corresponding fluorescence trace(s) will be removed form the graph.

Select the *Auto scale* option to scale the y-axis to accommodate the fluorescence data displayed.

To display the log of fluorescence vs. cycle number, select the *Log scale* option in the Data graph panel.



To expand regions of the graph, use the *X* and *Y* sliders to the left of the graph to zoom along the x and y axes. The scroll bars can be used to position the region of interest in the display panel. Alternatively, right click and drag a box around the area of the graph that you wish to magnify.

Moving the cursor over the data trace for a well will thicken the trace and display the C(t) value, along with the x and y coordinates corresponding to the current position of the cursor over the trace (see figure on page 8-8). Moving the cursor over a well in the Plate diagram or selecting a well in the Graphed Samples list will thicken the corresponding trace.

The *Smooth* slider bar, located at the top of the graph panel, allows the user to smooth the data display by creating a running average over a variable number of points. For example, 3-point smoothing takes each particular data point, along with the point before and after it, and takes an average of the three. This average is then plotted on the graph in place of the original data point. The default *Smooth* setting is 3. The user can turn off all smoothing by setting the slider all the way to the left. Smoothing of the data for each dye is done independently. In the Plate diagram panel, select Color *1*, *2*, *3*, or *4* to smooth the data for that particular dye.

Note: Smoothing the data can change the C(t) values.

Adjusting Data Analysis Options

After viewing a graph of the fluorescence data versus cycle number, you may wish to adjust some of the data analysis options. You can adjust the following data-analysis options in the Options panel of the Quantitation window:

- *Subtract blanks*: If this option is selected, for each cycle, the fluorescence measured in all wells designated as blanks (blue) will be averaged and subtracted from the fluorescence measured in all wells designated as samples (red) or quantitation standards (green).

- *Subtract baseline*: If this option is selected, the baseline signal, an absolute fluorescence value (or set of values in the case of the *Trend* option), will be subtracted from the fluorescence data collected in each well. This value is calculated based on the signals measured in each well and thus will vary from well to well.

  There are four options for defining the baseline signal value for a well:

  - *Minimum over cycle range*: The baseline signal value is defined as the minimum fluorescence value measured over the range of cycles specified in the *Cycle range* boxes.

  - *Average over cycle range*: The baseline signal value is defined as the average of the measured fluorescence calculated from the range of cycles specified in the *Cycle range* boxes.

  - *Global minimum*: The baseline signal is defined as the lowest fluorescence signal measured in the well, over all cycles. This is done for each well.

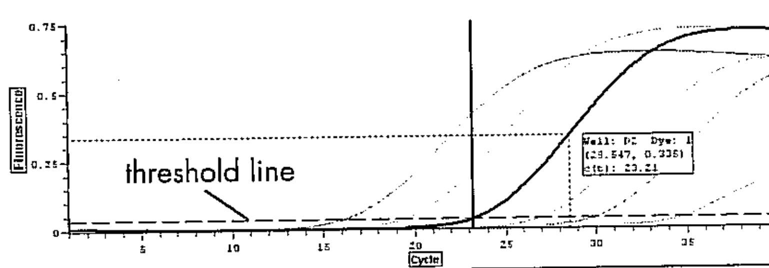
  - *Trend*: Opticon Monitor software looks for a trend of increasing or decreasing fluorescence and calculates a baseline to subtract from the signals that will eliminate this trend. When this option is selected, *Global minimum* is used to set the baseline for the data from cycles beyond the trend.

- *Suppress pre-threshold data*: This option can be used to specify the earliest cycle for which data is displayed on the Data graph. If the option is selected and the slider is positioned all the way to the right, the fluorescent data for selected wells will be plotted only for the cycle at which the threshold fluorescence is surpassed (the threshold cycle) and for subsequent cycles. Moving the slider to the left increases the number of cycles preceding the cycle threshold that are displayed. When the slider is positioned all the way to the left, the earliest cycle for which fluorescence data are plotted will be 10 cycles before the threshold cycle.

- Threshold: This quantitation option positions the threshold line, which defines each fluorescent curve's cycle threshold for use in quantitation of starting copy number (see "Adjusting the Cycle Threshold Line" immediately below).

Adjusting the Cycle Threshold Line

The threshold line, also known as the cycle threshold, or C(t) line, appears as a dashed horizontal line on the Data graph. Normally, this line should be positioned such that, on a graph of baseline-subtracted data, it intersects the fluorescence traces at a point where the signal intensities surpass background levels and begin to increase. To adjust the position of the threshold line, select one of the Threshold options, or left-click and drag the threshold line on the graph to the desired position. If no threshold line appears on the Data graph, select *Manual* from the Threshold options and enter a value for the threshold that is less than the maximum fluorescence value displayed on the y-axis of the graph. Then, drag the threshold line to the desired position.



Options for setting the threshold line include:

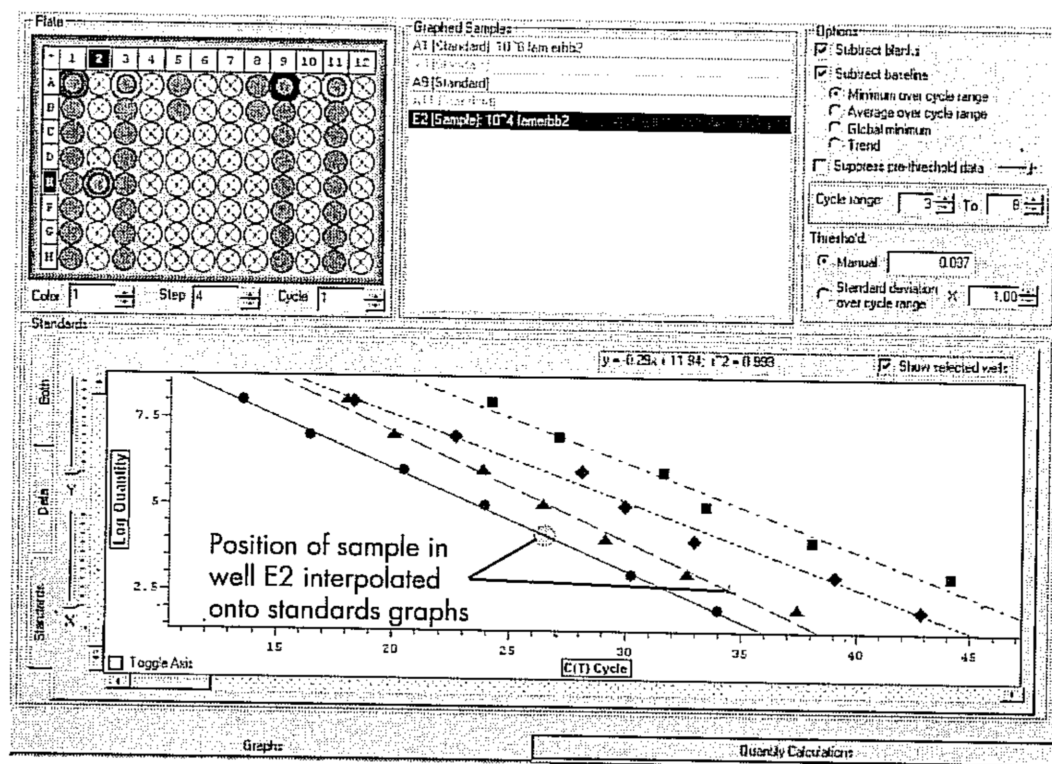
- *Manual:* The threshold line can be set manually by entering a threshold value for fluorescence intensity between 0–10, or by dragging the threshold line to the desired position on the graph.
- *Standard deviation over cycle range*: The threshold line can be set to a user-defined multiple of the standard deviation above the mean fluorescence across the Cycle range specified by the user (in the panel above the Threshold options). A maximum of 10 standard deviations is allowed.

It is often useful to consider both the Data and the Standards graphs (if standards were used) when establishing the position of the threshold line. The best option for setting the threshold line can often be determined by observing the effects of each option on the fit of the quantitation standards to a linear standard curve. The effect the position of the threshold line has on the standard curve can easily be visualized by dragging the line up and down.

In establishing the position of the threshold line, it may also be helpful to display the log of fluorescence vs. cycle number by selecting the *Log scale* option. Viewing the log of fluorescence may allow you to more accurately set the threshold line. In the log scale view, the threshold line should be placed in the linear portion of the curve, not low on the curve as it is in the fluorescence vs. cycle number view.

Standards Graph

To display a large Standards graph, click the *Standards* tab on the left-hand side of the graphs panel.



Standard curves are automatically generated using the information provided in the Specify Quantitation Standards screen during creation of the Plate file. The Standards graph(s) displays the base-10 logarithm of initial quantity (ng, moles, molecules, genome equivalents, copies, or user-defined units) versus the cycle threshold (C(t)) value, the cycle number at which the intensity trace intersects the threshold line. If Color *1* is selected in the field below the Plate diagram, the standards data and line equation for dye 1 will be displayed in the Standards graph. Similarly, if Color *2*, *3*, or *4* is selected, the standards data and line equation for the appropriate dye will be displayed in the Standards graph. The data for color 1 are plotted with circles, data for color 2 are plotted with triangles; for color 3, squares; and for color 4, diamonds. If the *All colors* option is selected, the standards data for the dye selected in the Colors field will be drawn with the symbol listed above fitted with a solid line, while the data for the other dyes will be drawn with their respecitve symbols fitted with a dotted line; the line equation for the dye selected in the Colors field will be the only line equation displayed. Note that *All colors* can only be selected when the Data graph is showing.

Written above the Standards graph is the equation describing the linear standard curve and the r-square value for the dye selected in the Colors box. To view the equation and r-square value for another dye, select a that dye in the Colors box.

The form of the equation is y = mx + b, where m is the slope of the line and b is the y-intercept. Theoretically, if perfect doubling of the template is obtained during each cycle, the slope of the line should be –0.30.

The r-square (r^2) value is the square of the correlation coefficient, often called the coefficient of determination. This number indicates what proportion of the variation in one variable is explained by variation in the other variable. In other words, the r-square value tells how close to the regression line the data points lie. The value of r-square can vary between 0 and 1, with values closer to 1 signifying a good fit. An r-square value of 0.999 indicates that the linear standard curve explains 99.9% of the variation in the data.

The *Toggle Axis* box in the bottom left corner of the Standards graph allows you to switch the x and y axes of the graph, if you have a preference for how the data are viewed. The default graph shows C(t) cycle on the x-axis and log quantity on the y-axis. Checking the Toggle Axis box switches the axes so that C(t) cycle is on the y-axis. Note that this also changes the r^2 value and the slope in the equation describing the standard curve.

Select the *Show selected wells* option to interpolate the Graphed Samples against the standard curve(s). The samples will be plotted with gray symbols fitted to the standard line (see figure on previous page).

If a Standards graph is not automatically displayed, check that the C(t) line has been appropriately set on the Data graph, and that the quantitation standards have been defined in the Specify Quantitation Standards screen (see the "Changing the Values of Quantitation Standards" section below).

Adjusting the Standard Curve

If desired, you can adjust the standard curve by deselecting outlying points. Moving the cursor over a data point will increase the size of the point and highlight the corresponding well in the Plate diagram, Graphed Samples list, and Data graph. To exclude a point from the standard curve, click on the point and it will turn red, indicating that it is no longer being used in the calculation of the curve. The standard curve will be automatically replotted to exclude the deselected point. Click the point again to include the point in the calculation of the curve; the point will again appear black.

If multiple sets of standards using the same dye have been included in a single run, points may be excluded so that the standard curves of interest are serially displayed. Recall that only the black (selected) standards are used in quantity calculations.

Changing the Values of Quantitation Standards

If, during creation of the plate file, a mistake was made in entering the values of quantitation standards, or quantitation standards were not specified, it is possible to change or add quantitation standards during the data analysis phase.

To change the values of the standards, first select the *Master* button on the toolbar. Select the *Edit* button in the Plate Setup panel and make the desired changes to the quantitation standards, as described in Chapter 6. Click *OK* to return to the Master File window, and then select the *Quantitation* button on the toolbar to continue analyzing data with the modified standards.

Quantity Calculations

Click the *Quantity Calculations* tab to display the Quantity Calculations screen.

Output

Well	Dye	Type	Label	C(T)	copies
B1	Run 1:FAM	Standard	10^6	16.370	000000.0000
B2	Run 1:FAM	Standard	10^6	16.270	000000.0000
B3	Run 1:FAM	Standard	10^6	16.351	000000.0000
C1	Run 1:FAM	Standard	10^5	19.726	100000.0000
C2	Run 1:FAM	Standard	10^5	19.391	100000.0000
C3	Run 1:FAM	Standard	10^5	19.094	100000.0000
D1	Run 1:FAM	Standard	10^4	23.330	10000.0000
D2	Run 1:FAM	Standard	10^4	23.695	10000.0000
D3	Run 1:FAM	Standard	10^4	23.532	10000.0000
E1	Run 1:FAM	Standard	10^3	27.029	1000.0000
E2	Run 1:FAM	Standard	10^3	27.127	1000.0000
E3	Run 1:FAM	Standard	10^3	27.636	1000.0000
F1	Run 1:FAM	Standard	10^2	30.310	100.0000
F2	Run 1:FAM	Standard	10^2	30.183	100.0000
F3	Run 1:FAM	Standard	10^2	30.424	100.0000
F5	Run 1:FAM	Sample	tumor RNA-multi	23.030	7845.6772
F6	Run 1:FAM	Sample	tumor ERBB2 single	23.642	0777.4605
F7	Run 1:FAM	Sample	tumor GAPDH single	None	N/A
H5	Run 1:FAM	Sample	tumor RNA multi	23.560	9241.9018
H6	Run 1:FAM	Sample	tumor ERBB2 single	23.294	10925.7842
H7	Run 1:FAM	Sample	tumor GAPDH single	None	N/A
B1	Run 2:VIC	Standard	10^6	21.333	000000.0000
B2	Run 2:VIC	Standard	10^6	20.650	000000.0000
B3	Run 2:VIC	Standard	10^6	20.302	000000.0000
C1	Run 2:VIC	Standard	10^5	24.002	100000.0000
C2	Run 2:VIC	Standard	10^5	24.940	100000.0000
C3	Run 2:VIC	Standard	10^5	24.412	100000.0000
D1	Run 2:VIC	Standard	10^4	28.350	10000.0000
D2	Run 2:VIC	Standard	10^4	27.625	10000.0000
D3	Run 2:VIC	Standard	10^4	28.508	10000.0000
E1	Run 2:VIC	Standard	10^3	31.265	1000.0000
E2	Run 2:VIC	Standard	10^3	32.453	1000.0000
E3	Run 2:VIC	Standard	10^3	31.988	1000.0000
F1	Run 2:VIC	Standard	10^2	35.129	100.0000

Well Selections

- Show all wells
- Show selected wells

Copy to Clipboard

Graphs | Quantity Calculations

The Quantity Calculations table lists:

- Well coordinates
- Assigned Dye
- The Type of well contents
- A descriptive well Label (if specified)
- The C(t) value, the cycle number at which the fluorescence intensity trace for a well intersects the threshold line on the Data graph.
- The initial quantity of template calculated to be present in the reaction.

To view the quantity calculations for only those wells selected in the plate diagram, choose the *Show selected wells* option. To view the quantity calculations for all non-empty wells, choose the *Show all wells* option.

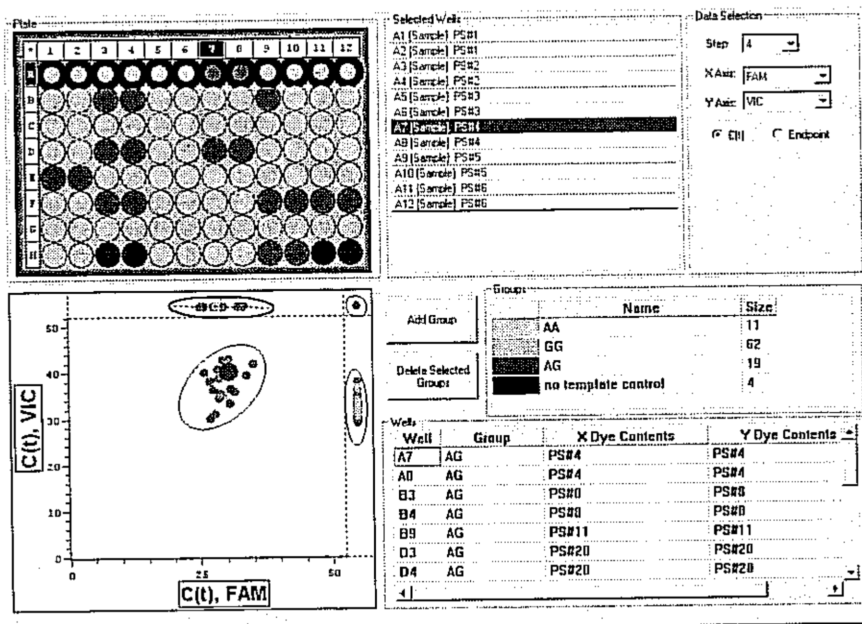
Click the *Copy to Clipboard* button to copy the quantity calculations to the clipboard for pasting into word processing or spread sheet programs.

Analysis of Fluorescence Data

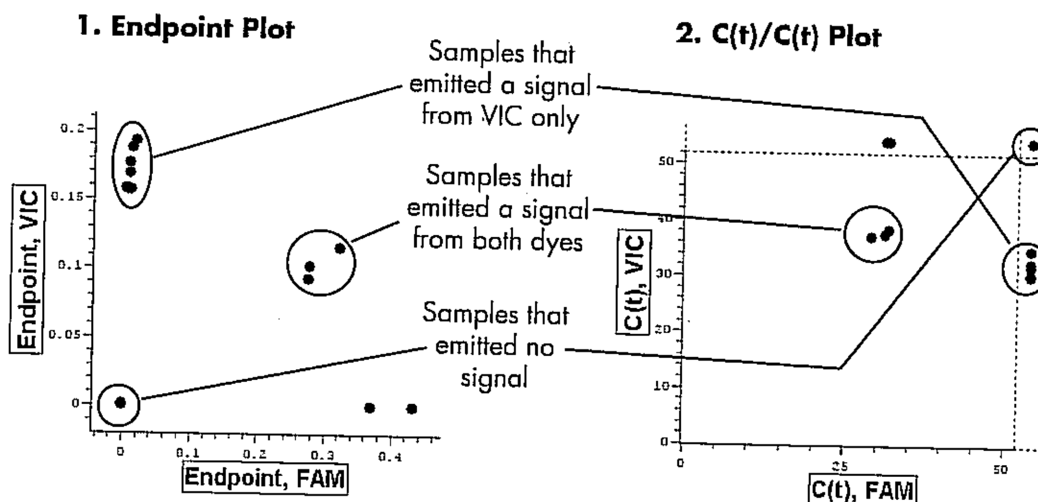
The Opticon Monitor software allows you to plot the fluorescent output of one dye against that of another; the resulting scatter plot can be used to identify samples that share similar C(t) or Endpoint signal values.

Creating a Scatter Plot

If a run is in progress or has just completed, click the *Analysis* button on the toolbar to access the Analysis screen. To access a previously generated data file, select *Open* and then *Data File* from the *File* menu. Once the data file loads, click the *Analysis* button on the toolbar.



In the Data Selection panel, select which protocol step you want to analyze, which dye you want displayed on each axis, and whether you want to compare the fluorescence data based on *C(t)* or *Endpoint*.



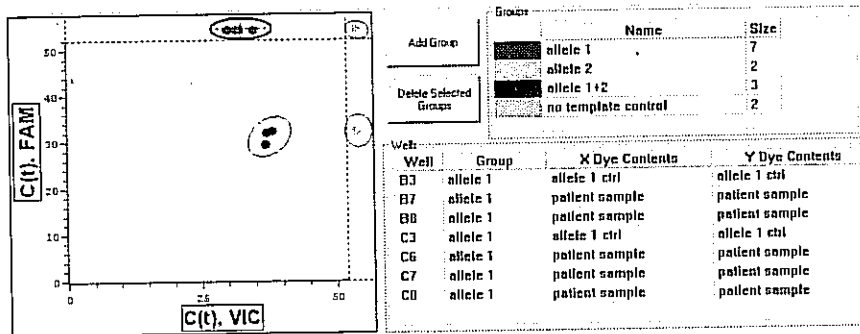
1. *Endpoint* plot: The graph on the left plots the endpoint fluorescence data from each well in a two-color experiment, with the fluorescence in the FAM channel plotted along the x-axis and the fluorescence from the VIC channel plotted along the y-axis. The software has plotted the relative fluorescent intensities for each dye, as measured at the end of the run.
2. *C(t)/C(t)* plot: The graph on the right plots the C(t) values obtained in the same experiment as in the graph on the left. The C(t) for FAM is plotted along the x-axis and the C(t) for VIC is plotted along the y-axis. The dashed lines at the top and right edges of the graph are separators that mark the last cycle of the experimental protocol. The fluorescence of those samples plotted above or to the right of these lines did not rise above the C(t) line during this experiment.

As the circles in the above illustration show, these plots naturally sort well data into groups defined by which dyes emitted above-background signals: the x-axis dye, the y-axis dye, both dyes, or neither dye. Such plots are useful for assigning genotypes to particular samples or groups of samples, in experiments in which each of two alleles is labeled with a different dye.

Defining Subsets of Data

From the scatter plot, the user is able to identify subsets of data based on the clustering pattern of the data points. For example, in genotyping, samples with the same genotype will tend to cluster in a similar C(t) range. Users can select samples that cluster together, and assign particular group names, e.g. the genotype, to the samples located in these clusters.

To group a cluster of data points, click on the *Add Group* button in the center of the Analysis window. This converts the mouse pointer to a set of cross hairs. Move the cross hairs to the periphery of a clustered data set. Left-click the mouse to set an anchor point on one side of the data set, drag the cross hairs to the opposite edge of the data set, and left-click the mouse to mark the end of the set. Dragging the cross hairs perpendicular to the ellipse that was just created causes the ellipse to radiate outward. Expand or contract the radius to include the data points that you wish to analyze. Data points turn from black to a particular color (red, green, blue, etc.) when they are included in a set. When you are satisfied with the area that you have defined, left-click the mouse a final time to set the group.



There are two ways to alter the ellipse after it has been drawn. To change the dimensions of the ellipse, click on the one of the four points that lie directly on the ellipse. (These will appear when you position the mouse pointer on the ellipse). Dragging these points can alter both the shape and radius of the ellipse. To move the ellipse without changing its dimensions, click on the periphery of the ellipse and drag it to a new location.

When a group has been created, it will be listed in the Groups panel as a "New Group", along with the number of data points that are in the group. To rename the group, highlight the group name, type the new name and then press *Enter*. To delete a group, highlight the group name and then click the *Delete Selected Groups* button.

A defined group will be color coded—the data points included in the set will be assigned a color. Sample wells that correspond to these data points will turn this same color in the Plate diagram. Likewise, this color scheme will appear in the Groups panel to identify all the linked data points. You can change the color of a group by highlighting its name in the Groups panel then clicking the colored box to the left of the name. Select a different color from the palette that appears.

When a group is selected (either by clicking on a group name from the Groups panel or an ellipse in the scatter plot), its contents and sample description will be listed in the Wells panel.

Identifying Samples in the Endpoint and C(t) Plots

If no wells have been selected in the Plate diagram, all the points in the scatter plots appear either the color corresponding to their assigned group, or black, if they have not been assigned to a group. If a well is then selected in the plate diagram, the color of its corresponding data point in the scatter plot remains the same, whereas all other data points turn gray. If you now click on another well in the plate diagram, the point corresponding to the newly selected point will become colored according to its group membership (or black if it has not been assigned to a group). The originally selected cell will become deselected and turn gray unless you press the control key while clicking on the additional well (see the section on "Selecting and Deselecting Wells" in chapter 6). Clicking the "*" in the upper-left corner of the Plate diagram will select all wells, thus returning them to the color(s) they were when no wells were selected.

By positioning the mouse above particular data points in the scatter plot, you can identify the corresponding samples on both the Plate diagram, and in the Selected Wells panel (if those wells have been selected in the Plate diagram).

Exporting and Printing Quantitation and Analysis Data

For information on exporting and printing quantitation data, refer to the "Exporting and Printing Data" section near the end of this chapter.

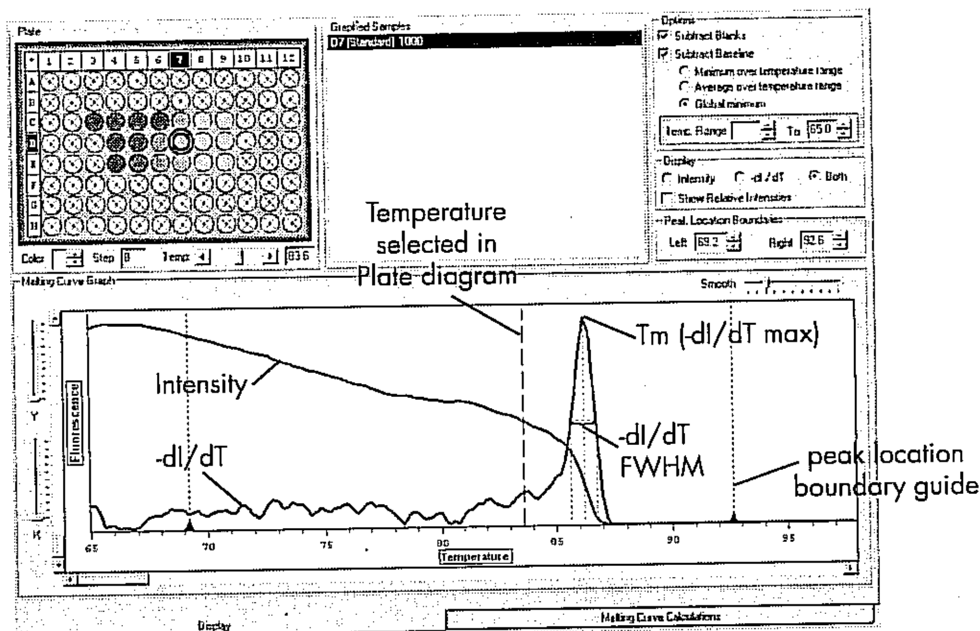
Melting Curve

A melting curve analysis can aid in product identification and determination of product homogeneity, often eliminating the need for time-consuming electrophoresis. If a chemistry's fluorescence is dependent on DNA annealing, a decrease in fluorescence is observed as melting progresses. Because the melting temperature of nucleic acids is affected by length, G+C content, and the presence of base mismatches, among other factors, products can often be distinguished by their melting characteristics.

If a run is in progress or has just completed, click the *Melting Curve* button on the toolbar to analyze the melting curve data. To access a previously generated data file, select *Open* and then *Data File* from the *File* menu. Select the desired file from the standard browse window. To view a recently created data file, select the desired file from the *Recent Data Files* list accessible from the *File* menu. Once the data file loads, click the *Melting Curve* button on the toolbar to analyze melting curve data.

Display

The Display screen is the default screen in the Melting Curve analysis window. In this screen, you can display a graph of Fluorescence versus Temperature, –dI/dT versus Temperature, or both, for wells selected in the Plate diagram.



1. Use the Plate diagram to select the wells to be included in the graph. See the "Selecting Wells Using the Plate Diagram" section in Chapter 6 for additional information.

   Selected wells appear outlined in color. The color outlining the well corresponds to the color of the well coordinates in the Graphed Samples list and to the color of the trace in the Melting Curve Graph.

   Deselect all wells by clicking on any selected or empty well. To deselect a subset of wells, hold down the control key, and click on the well(s) you wish to deselect. The well(s) will no longer appear outlined in color, and the corresponding trace(s) will be removed from the graph.

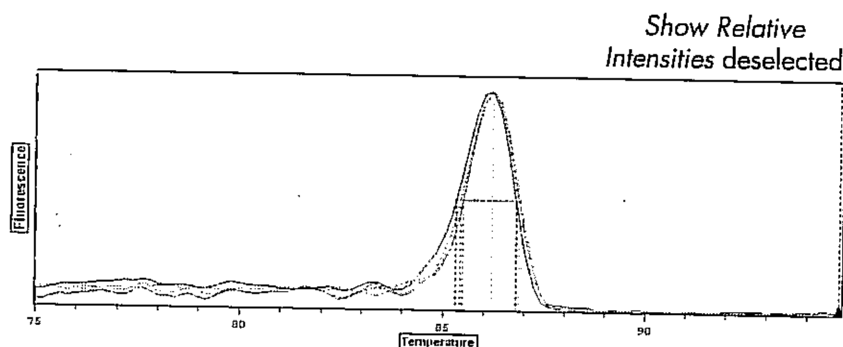
2. Select Color *1* in the Plate diagram to display the melting curve data for dye 1 for all of the selected wells. Select Color *2*, *3*, or *4* to display the melting curve data for the respective dyes for all of the selected wells.

3. The Temp. field can be used to highlight the results associated with a particular temperature, by drawing a dotted vertical line on the graph at the designated temperature. Either use the scroll bar to scroll to the desired temperature, or enter the desired temperature in the box located to the right of the scroll bar.

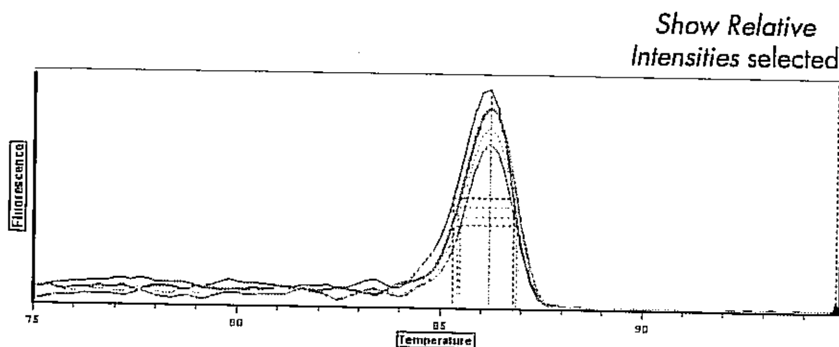
4. Use the Display panel to choose how the melting curve data are graphed.

   - Select *Intensity* to graph fluorescence intensity versus temperature.
   - Select *–dI/dT* to graph the negative first derivative of the fluorescence intensity versus temperature. The dotted vertical line, drawn in the same color as the corresponding trace, marks the maximum –dI/dT value, the temperature at which the rate of change in fluorescence is the greatest. This is defined as the melting temperature (T_m) of the product. The dotted horizontal line indicates the sharpness of the –dI/dT curve as the number of degrees Celsius over which the curve spans (i.e., the curve width) at half of the maximum –dI/dT value calculated for the well (i.e. the FWHM, or full width half max).
   - Select *Both* to simultaneously display the Intensity and the –dI/dT graphs.
   - Select *Show Relative Intensities* to display the relative intensities of the signals for the selected wells. If this option is not selected, the melting curves of all wells will be automatically scaled so that the peaks are the same height.

Show Relative Intensities deselected



Show Relative Intensities selected



5. After viewing a graph of the fluorescence data versus temperature, you may wish to adjust some of the data analysis options. You can adjust the following data analysis options in the Options panel of the Melting Curve window:

   - *Subtract Blanks:* If this option is selected, the fluorescence measured in all wells designated as blanks (blue) will be averaged and subtracted, as background, from the fluorescence measured in all wells designated as samples (red) or quantitation standards (green).

   - *Subtract Baseline*: If this option is selected, the baseline signal, an absolute fluorescence value, will be subtracted from the fluorescence data collected in each well. This value is calculated based on the signals measured in each well and therefore will vary from well to well.

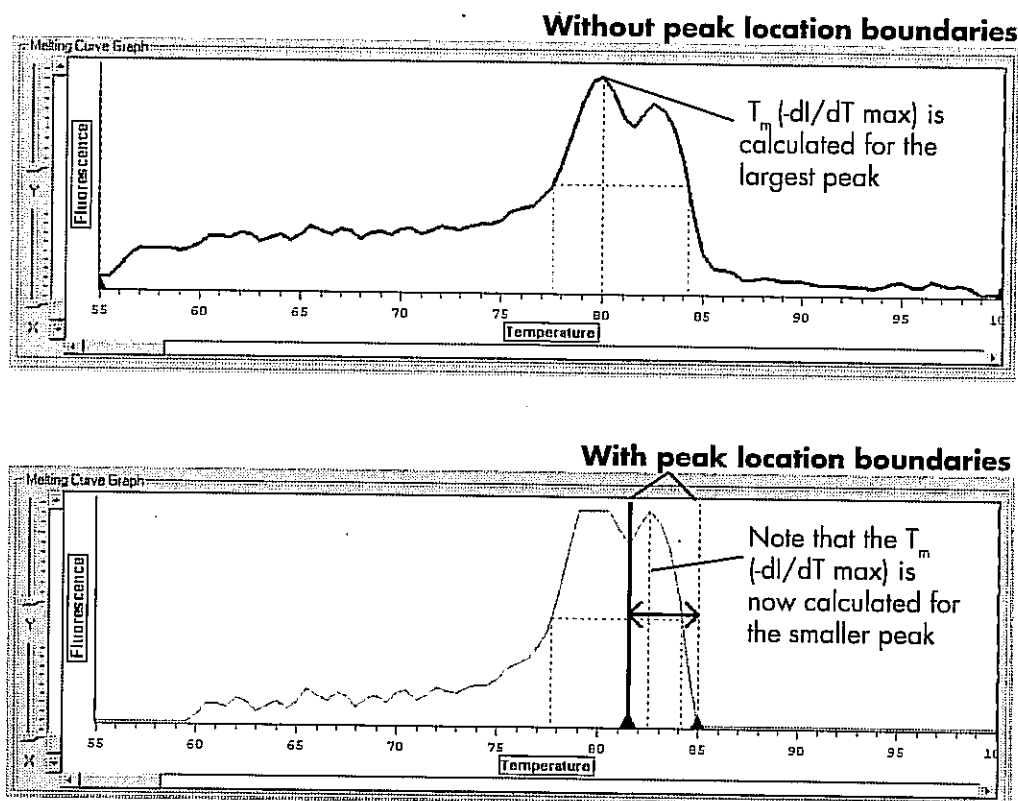
     There are three ways to define the baseline signal value for a well:

     - *Minimum over temperature range*: The baseline signal value is defined as the minimum fluorescence value measured in the range of cycles specified in the Temp. Range field.

     - *Average over temperature range*: The baseline signal value is defined as the average of the measured fluorescence calculated from the range of cycles specified in the Temp. Range field.

     - *Global minimum*: The baseline signal is defined as the weakest fluorescence signal measured in the well. This value will be set to zero.

6. The Peak Location Boundaries panel allows you to limit the range of temperatures over which Opticon Monitor software looks for peaks in the –dI/dT trace, thereby limiting the range of possible calculated melting temperatures. This can be particularly useful for excluding unwanted peaks, or for determining the melting temperature for a second, smaller peak (e.g., when genotyping heterozygotes). You can set Left and Right peak location boundaries by entering the temperature or by using the arrows to scroll to the desired temperature. Alternatively, drag the peak location boundary guides to the desired location on the graph.



7. The *Smooth* slider allows you to change the number of points that are used in calculating a running average to smooth the melting curve graph. This can be particularly useful for resolving peaks when many reads have been collected over small increments in temperature, resulting in a choppy graph. The default setting for well-resolved data is 3.

Melting Curve Calculations

Click on the *Melting Curve Calculations* tab at the bottom of the Melting Curve window to display the melting curve calculations screen.

Output

Well	Dye	Type	Label	Tm (-dI/dT max)	dI/dT FWHM
B3	Run 1:SBG1	Standard	10000 copies hu genomic DNA	89.4	2.18
B4	Run 1:SBG1	Standard	1000 copies hu genomic DNA	89.6	2.17
B5	Run 1:SBG1	Standard	100 copies hu genomic DNA	89.2	2.20
B6	Run 1:SBG1	Standard	100 copies hu genomic DNA	89	2.35
B7	Run 1:SBG1	Standard	1 copy hu genomic DNA	89.0	2.26
B8	Run 1:SBG1	Sample	Unknown sample	88.4	1.79
B9	Run 1:SBG1	Blank		75	0.39
C3	Run 1:SBG1	Standard	10000 copies hu genomic DNA	89.8	1.94
C4	Run 1:SBG1	Standard	1000 copies hu genomic DNA	89.4	2.15
C5	Run 1:SBG1	Standard	100 copies hu genomic DNA	89.4	2.31
C6	Run 1:SBG1	Standard	100 copies hu genomic DNA	89.6	2.69
C7	Run 1:SBG1	Standard	1 copy hu genomic DNA	84	2.29
C8	Run 1:SBG1	Sample	Unknown sample	62	0.59
C9	Run 1:SBG1	Blank		83	0.60
D3	Run 1:SBG1	Standard	10000 copies hu genomic DNA	89.6	1.84
D4	Run 1:SBG1	Standard	1000 copies hu genomic DNA	89.6	2.12
D5	Run 1:SBG1	Standard	100 copies hu genomic DNA	89	2.04
D6	Run 1:SBG1	Standard	100 copies hu genomic DNA	89	2.07
D7	Run 1:SBG1	Standard	1 copy hu genomic DNA	89.4	2.42
D8	Run 1:SBG1	Sample	Unknown sample	86.0	1.03
D9	Run 1:SBG1	Blank		81.8	0.86

Copy to Clipboard

Display | Melting Curve Calculations

The melting curve calculations table lists:

- Well coordinates
- Dye used
- The Type of well contents (blank, standard, etc.)
- A descriptive well Label, if entered during creation of the plate file
- Tm (–dI/dT Max): The melting temperature (T_m), the temperature at which –dI/dT is at the maximum value calculated for the well.
- dI/dT FWHM (Full Width Half Maximum): The width of the curve, in degrees Celsius, at half of the maximum –dI/dT value calculated for the well; describes the sharpness of the –dI/dT peak

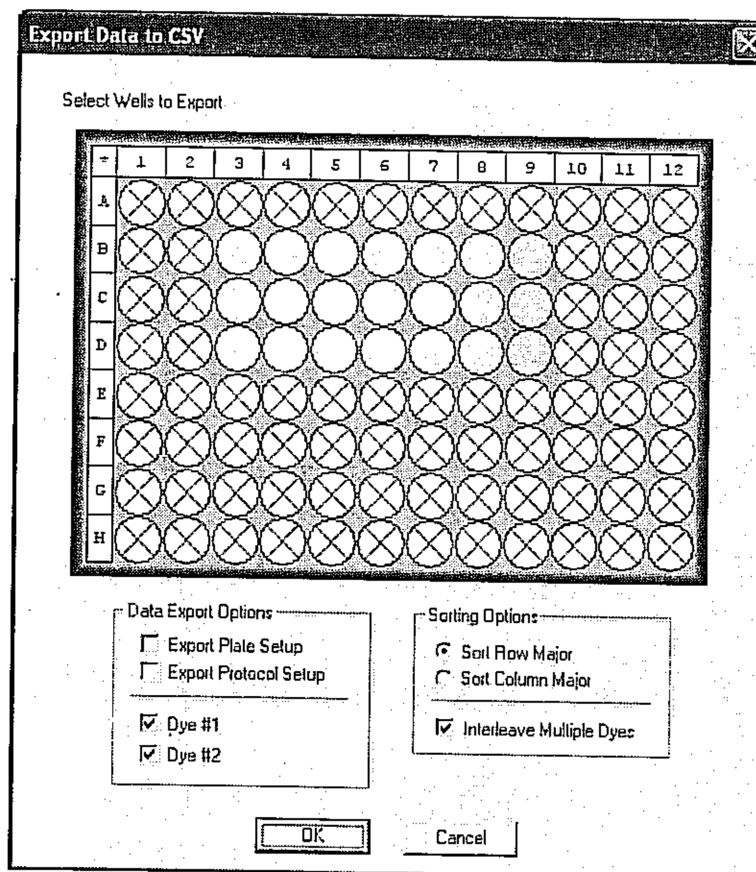
Click the *Copy to Clipboard* button to copy the melting curve calculations to the clipboard, for pasting into word-processing or spreadsheet programs.

Exporting and Printing Data

Export

For customized data analysis, Opticon Monitor software provides the option to write the fluorescence data collected during a run to a comma-separated-values (CSV) file, readable by Microsoft Excel. The Export command creates a CSV file listing all fluorescence measurements (for selected wells) that were collected during all plate read and melting curve steps in an experimental run.

To export data for selected wells, choose *Export* (under the *Quantitation, Melting Curve,* or *Analysis* menu, depending on which window is being viewed). Wells can be selected in the window that appears.



The user can also select the dye(s) for which data will be exported, by selecting *Dye #1, Dye #2, Dye #3,* and/or *Dye #4*. The user can choose to sort the wells by row or by column; and can choose whether to *Interleave Multiple Dyes* (i.e. to list data for all dyes in each well together), or to first list the data for each well for the first dye, then list the data for each well for the second dye, and so forth.

In addition to exporting fluorescence data, the user can choose to export the plate and/or protocol setup information by checking *Export Plate Setup* and/or *Export Protocol Setup*. The plate information that is exported includes the well number, dye used, well contents, and well descriptions. If the plate setup is exported, the information can later be pasted into a different master file to easily reconstruct the plate file (see "Pasting from Microsoft Excel" in Chapter 6).

By default, the exported CSV file also lists analysis options (i.e. Subtract Blanks, Subtract Baseline, Baseline Start cycle, Baseline End cycle, and Suppress Pre-Threshold) that were chosen in the Analysis window for each protocol step at which data were collected.

Copy to Clipboard

The *Copy to Clipboard* option (under the *Quantitation, Melting Curve,* or *Analysis* menu, depending on which window is being viewed) allows the user to paste run information and graphical data into a variety of applications for presentation or storage. The following items can be copied using *Copy to Clipboard* from the specified windows:

From the Quantitation Window

The *Data Graph* (a plot of fluorescence—or log fluorescence—against C(t) cycle), *Standards Graph, Microtitre plate* (the Plate diagram), or *Quantity calculations* can be copied to the clipboard and pasted into several applications. Selecting *Quantity Calculation* from *the Copy to Clipboard* menu generates a CSV table to be pasted subsequently into an Excel spreadsheet. The table has the same content as the Quantity Calculations screen: for each well in the experiment, the table lists the name of the sample, the fluorescent dye used, the well contents (i.e., blank, standard, or sample), the C(t) value, and the specified (for standards) or calculated (for samples, by interpolation against the standard curve) quantity of template present in the well at the initiation of amplification. This table can also be copied to the clipboard by clicking on the *Copy to Clipboard* button on the Quantity Calculations screen.

From the Melting Curve Window

The *Data Graph* (the plot of fluorescence intensity or –dI/dT or both), *Microtitre plate* (the Plate diagram) or *Melting Temp Calculations* can be copied to the clipboard. Selecting *Melting Temp Calculations* generates a CSV file with information identical to that shown on the Melting Curve Calculations screen: well coordinates, dye used, well content (i.e., blank, standard, or sample), Label, if one was given, T_m (–dI/dT Max), and dI/dT FWHM (see the section on "Melting Curve Calculations" for more details of this table). This table can also be copied to the clipboard by clicking on the *Copy to Clipboard* button on the Melting Curve calculations screen.

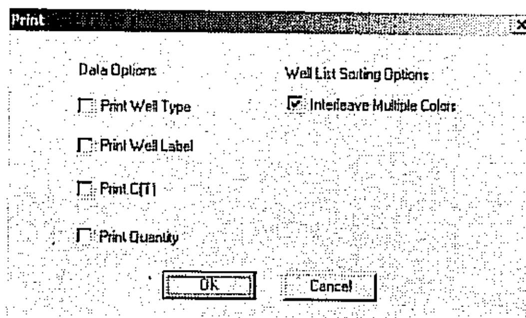
From the Analysis Window

The *Data Graph* (the plot of C(t) or Endpoint fluorescence), *Microtitre Plate,* or *Analysis List* can be copied to the clipboard. Selecting *Analysis List* generates a CSV file listing the Group Name, Well, Label (if previously specified by the user), and the Dye contents (standard, sample, etc.) for each sample contained by a Group in the scatter plot (as defined by the user — see section on "Defining Subsets of Data").

Print

Opticon Monitor software can assemble a report of the real-time run and send it directly to a networked printer. This can be done from either the Quantitation window or the Melting Curve window.

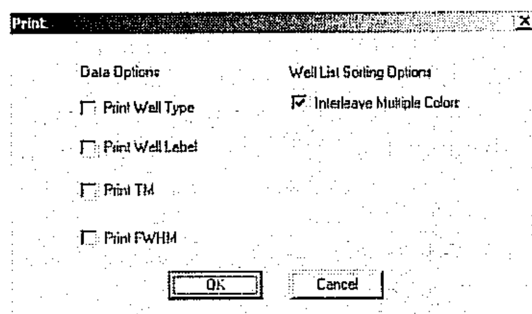
If *Print* is selected from the *Quantitation* menu, the software creates a report that contains: the user's name; the name and location of the data file on the Opticon Monitor computer's hard drive; an indication of the active color; the position of the C(t) line; and an indication of whether the C(t) level was set manually or "via signal-to-noise". The report will also include the Plate diagram with selected wells highlighted; the Data graph of fluorescence (or log fluorescence) versus C(t) cycle; and the Standards graph along with the equation detailing the fit of the data. Finally, the report will include a table listing which wells were selected. In addition, the user can select other options to be included in the table, from the window that appears after *Print* is selected:



The Data Options list allows the user to incorporate the following additional features into the table presented in the report: *Well Type* (sample, standard or blank), *Well Label* (the contents of the well as entered by the user), the *C(t)* for each individual sample, and *Quantity* (the starting copy number of each quantitated DNA species in each well).

The Well List Sorting Options box allows the user to select how the data will be sorted. By default, *Interleave Multiple Colors* is selected (i.e., well A1, dye 1; well A1, dye 2; well A1, dye 3; well A1, dye 4; well A2, dye 1; well A2, dye 2; well A2, dye 3; well A2, dye 4; etc.). To sort by dye (i.e., well A1, dye 1; well A2, dye 1; well A3, dye 1; etc.), deselect this option.

If *Print* is selected from the *Melting Curve* menu, the software creates a report that contains: the user's name; the name and location of the data file on the Opticon Monitor computer's hard drive; and an indication of Display Type, i.e., whether intensity, –dI/dT, or both are plotted in the graph. The report will also include the Plate diagram with selected wells highlighted; and the Melting Curve Graph of fluorescence intensity and/or –dI/dT vs. temperature. Finally, the report will include a table listing the wells that are plotted. In addition, the user can select other options to be included in the table, from the window that appears after *Print* is selected:



The Data Options list allows the user to incorporate the following additional features into the table presented in the report: *Well Type* (sample, standard or blank), *Well Label* (the contents of the well as entered by the user), The melting temperature, *TM* , for each individual sample, and *FWHM* (the full width of the melting curve peak, at half it's maximum height).

The Well List Sorting Options box allows the user to select how the data will be sorted. By default, *Interleave Multiple Colors* is selected (i.e., well A1, dye 1; well A1, dye 2; well A1, dye 3; well A1, dye 4; well A2, dye 1; well A2, dye 2; well A2, dye 3; well A2, dye 4; etc.). To sort by dye (i.e., well A1, dye 1; well A2, dye 1; well A3, dye 1; etc.), deselect this option.

Create HTML Report

For users interested in publishing their results on the web, Opticon Monitor software will generate a folder that is HTML-ready. This folder includes all .bmp versions of graphical images as well as the HTML code and syntax for posting the data report. Select *Create HTML Report* from the *Quantitation* or *Melting Curve* menu, and you will be presented with a window similar to the one above. Again, you will be allowed to include the well type, well label, C(t) value, and initial DNA quantity for each sample, as well as the ability to sort these data by well or by dye.

9. Maintenance

Cleaning the Chromo4 Unit, 9-2
  Cleaning the Chassis and Block, 9-2
  Cleaning the Air Vents, 9-2
  Cleaning the Optics, 9-2

Cleaning the Chromo 4™ Unit

Cleaning the Chassis and Block

Clean the outside of the Chromo4 unit with a damp, soft cloth or tissue whenever something has been spilled on it, or when the chassis is dusty. A mild soap solution may be used if needed.

Clean block wells with swabs moistened with water, 95% ethanol, or a 1:100 dilution of bleach in water. If using bleach, swab wells with water afterward to remove all traces of bleach. Clean spilled liquids out of the block as soon as possible: dried fluids can be difficult to remove. Do not clean the block with caustic or strongly alkaline solutions (e.g., strong soaps, ammonia, or bleach at a higher concentration than specified above). These will damage the block's protective anodized coating, and possibly lead to electrical shorting.

Caution: Do not pour any cleaning solution into the block's wells and then heat the block, in an attempt to clean it. Severe damage to the block, the heated lid, and the chassis can result.

Cleaning the Air Vents

With the Chromo4 detector and DNA Engine® cycler turned off, clean the air intake and exhaust vents with a soft-bristle brush, a damp cloth, or a vacuum cleaner whenever dust is visible (see figure 2-1). If these vents become clogged with dust and debris, airflow to the heat sink will be hampered, causing performance problems related to overheating.

Tip: To prevent problems with overheating, check regularly for dust buildup.

Cleaning the Optics

The optical components of the detector should not be cleaned by the user.

Should you suspect difficulty with the optics, please contact the customer service staff of MJ Research at 888-652-9253, or your local distributor.

10. Troubleshooting

Calibration, 10-2
Testing Calibration, 10-2
Recalibrating, 10-2
Software Error Messages, 10-6

Calibration

The Chromo4™ detector is calibrated at the factory to accommodate eleven of fluorescent dye chemistries (SYBR Green I, FAM, TET, HEX, VIC, JOE, TAMRA, ROX, Texas Red, Cy3, and Cy5) and two microtiter plates (white wells and clear wells). Calibrations may be added, edited or removed via the *Dye Calibration* interface from the *Tools* menu. However it is strongly recommended that the factory calibrations not be removed or changed as this may adversely affect the performance of the instrument.

Testing Calibration

An accurately calibrated instrument will detect the same fluorescence intensity in every well when reading samples of uniform composition.

To determine if the Chromo4 detector is accurately calibrated:

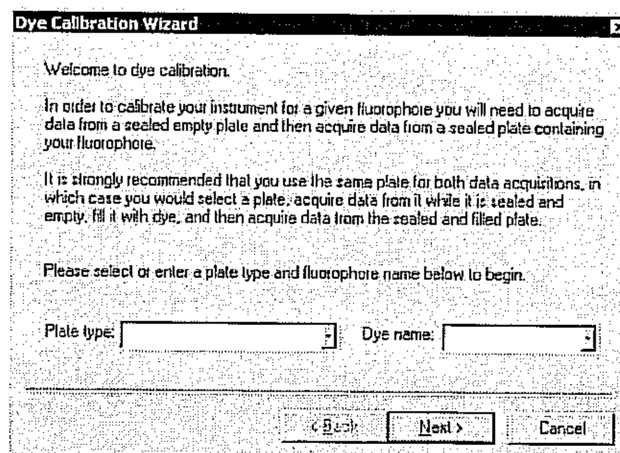
1. Prepare a test plate by accurately pipetting 50µl of a carefully prepared dye solution into each of the 96 wells of a microplate.
2. Program the following protocol:
    - Temperature step of 30°C for 30 seconds
    - Plate Read step
    - Goto line 1 for a total of 2 more times
    - End.
3. Analyze the fluorescence data for all 96 wells using the Optical Read Status screen. If the instrument is accurately calibrated, the fluorescence data should appear as relatively straight, horizontal lines that are tightly clustered—within the accuracy of pipetting. Several test plates should be measured to determine the error resulting from pipetting.
4. If the fluorescence data do not appear as tightly clustered straight lines, follow the instructions below for recalibrating the instrument.

Recalibrating

1. Select *Dye Calibration* from the *Tools* menu.
2. The Dye Calibration Wizard will launch and provide basic instructions for creating new calibrations.
3. Dye calibrations are performed by first scanning a blank plate (an empty microplate that is sealed with clear optical caps), followed by scanning the same plate containing the fluorophore of interest (also sealed with clear optical caps). Differences in the collected signals from the "dye plate" and the "empty plate" are used to determine color separation matrices that resolve the dye of interest from mixtures. It is strongly

recommended that the same plate be used for both the "empty" plate read and the "dye" plate read, as variations from plate to plate can be significant and interfere with obtaining an optimal color separation matrix.

4. Follow the instructions provided by the Wizard and begin by selecting or entering a *Plate type* into the appropriate field on the first screen. This plate type description will identify the type of microplate used for this particular calibration (e.g., White, Clear).

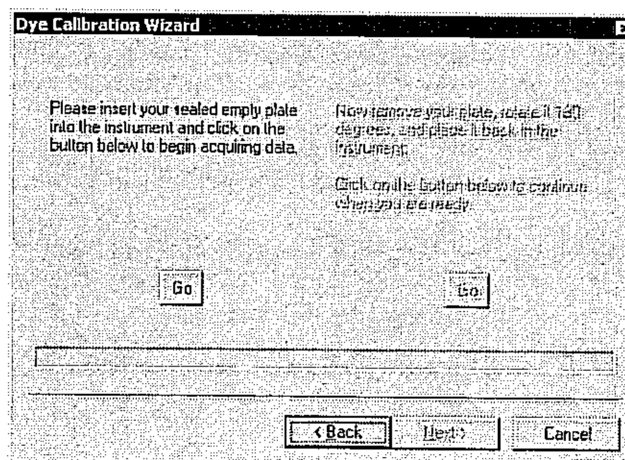


5. After selecting a plate type, enter the dye to be calibrated or select the dye from the *Dye name* drop-down list (e.g., FAM, VIC).

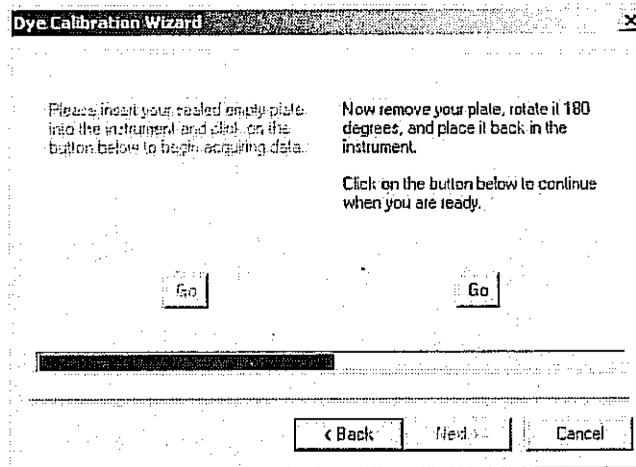
6. Proceed to the next screen by clicking on the *Next >* button.

7. If a calibration already exists for this particular dye/plate combination, a warning message will appear. If you wish to continue, click on the *Yes* button.

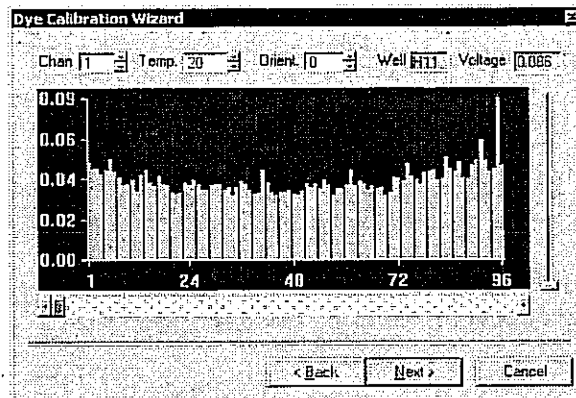
8. Insert the sealed empty plate, close the door and click on the *Go* button. The Chromo4 system will begin the calibration procedure.



9. Remove the plate and rotate it 180 degrees when prompted. Close the door and click on the *Go* button to continue.



10. After the empty plate has been scanned, you will be able to view the acquired data and check it for anomalies that may indicate a problem with the plate (i.e., unusually high signal in certain wells). See Example below:



Note that you can view the results for different channels, temperatures, orientations and wells

11. Click on the *Next* > button to proceed to the dye plate section.

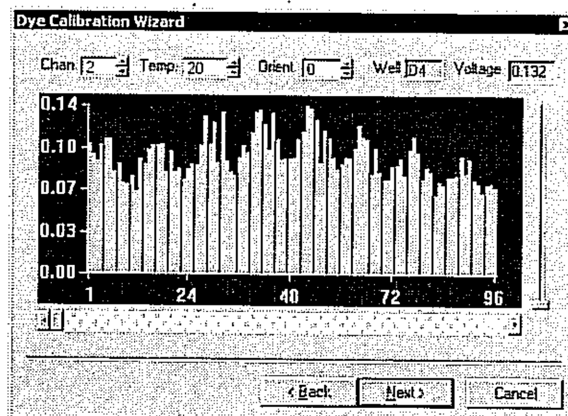
12. Remove the empty plate from the Chromo4 instrument. Remove the optical caps and fill each well with 50µl of dye diluted in reaction buffer. The fluorophore should be present at a relatively high concentration to insure the signal obtained is largely from the dye itself.

13. Seal the filled dye plate with clear optical caps and place the plate inside the Chromo4 instrument.

14. Click on the *Go* button to continue the calibration.

15. Remove the plate and rotate it 180 degrees when prompted. Close the door and click on the *Go* button to continue.

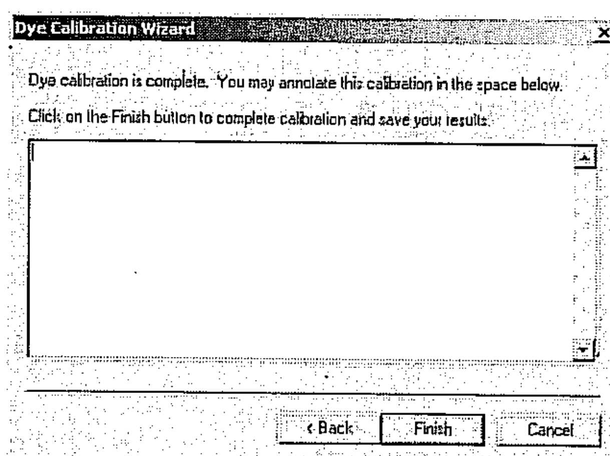
16. After the dye plate has been scanned, you will be able to view the acquired data and again check it for anomalous readings. Ideally, the average signal on at least one of the channels should be at least 10X greater than the blank plate signals for that channel. This insures that the dye is providing the vast majority of the total signal and will insure a robust color separation matrix.



17. Use the fields above the bar graph to select which channels to view. The graph will display the fluorescence readings for the dye plate. These values should be very consistent with respect to one another. If this is not the case, you may need to redo the dye plate.

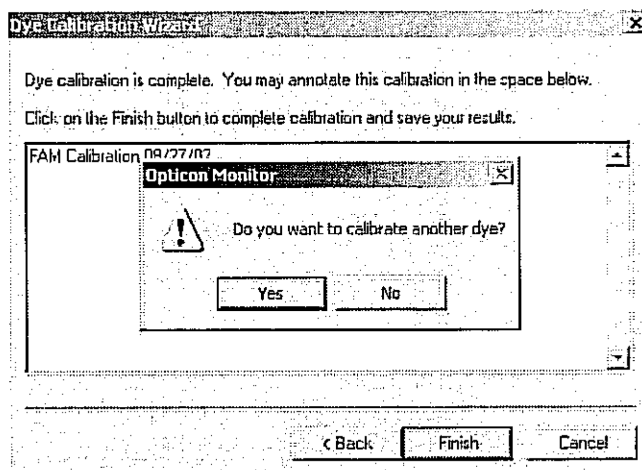
18. If the results are satisfactory, click on the *Next* > button to proceed to the final screen.

19. Add comments (if desired) to the calibration (e.g., record the date, or other conditions).

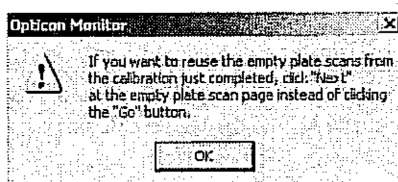


20. Click on *Finish* to save and complete the calibration process.

21. To run another calibration with the same plate type, click the *Yes* button when the Opticon Monitor software asks if you want to calibrate another dye.



22. You will then be instructed to click the *Next* button at the empty plate scan page instead of clicking the *Go* button. This will allow you to recycle the empty plate scan you took earlier. (Repeat steps 16-19.)



To run another calibration with a different plate type, close the wizard and restart the calibration process from the beginning.

Software Error Messages

The following tables list software error messages along with their probable causes and suggested resolutions. For help resolving software problems or for additional information, contact MJ Research technical support at 888-652-9253 (in the US or Canada) or contact your local distributor (outside the US or Canada).

Error Message	**Cause**	**Resolution**
Opticon Monitor must run at a screen resolution of at least 1024 x 768. Please adjust your resolution and restart Opticon Monitor.	The resolution of the computer monitor is set too low.	Increase the resolution and restart the Opticon Monitor software.
The protocol exited with a failure indication.	Opticon Monitor could not access the thermal cycler to start/run a protocol	Check power and serial cable connections
The Chromo4 is not responding. Please check that all communication and power cables are secured, the power switch is in the on position and the door is closed.	Opticon Monitor could not communicate with the Chromo4.	Check that the door is closed. Check power and serial connections.
The load was aborted because the file contains improper or corrupted data.	The data file has been damaged in a way that Opticon Monitor cannot open it.	Contact MJR Technical Support.
The door is open. Please close it and click OK to continue; click Cancel if you do not wish to start the protocol now.	A protocol was commenced with the door open.	Shut the door completely and try again.
Opticon Monitor seems to be running a protocol. Are you sure you want to exit?	User attempted to exit Opticon Monitor while a run was in progress.	Either cancel the exit to continue the run, click the Stop button to stop the run, or exit leaving the protocol running.
Your protocol is empty. You must set up a protocol before continuing.	User attempted to start a run with no protocol.	Create or open a protocol file and try again.
Dye file(s) on plate <PLATE> don' t exist. Please fix these dyes : <DYE1>, <DYE2>. Yes to fix dyes, No to continue with identities in place of dyes, Cancel to cancel.	The plate setup contains dyes that do not exist on the computer.	Clicking YES will give the user the opportunity to choose different dyes. Clicking NO will allow the user to continue with the run using id1 and id2 (non-color separated).Cancel will cancel the run.
Your username cannot have the character "<CHAR>" in it. Please choose a new username.	The following characters are invalid for file names: \/:*?"<>|.	Change username so that illegal characters are not used.

Error Message	**Cause**	**Resolution**
<FILENAME> The above file name is invalid.	User tried to save a file with illegal characters in it.	Change file name so that illegal characters are not used.
Please correct the plate type and dye name as follows: * The current dye name contains one of: \/" *?" <> I _ Please choose a name that doesn' t use any of these characters.	User tried to save a dye file with illegal characters in it.	Change dye name so that illegal characters are not used.
Please correct the plate type and dye name as follows: * The current plate type contains one of: \/" *?" <> I _ Please choose a name that doesn' t use any of these characters.	User tried to save a plate type with illegal characters in it.	Change plate type name so that illegal characters are not used.
Please close the instrument' s door.	The door was opened during a calibration pass.	Close the door and continue.
Would you like to proceed to the next protocol step? (Skipping in a loop or during a melting curve skips the rest of the loop or melting curve)	User pressed the skip button during a protocol.	Opticon Monitor cannot skip steps inside a quantitation loop or a melting curve. Rather it will skip to the first protocol step outside the current quantitation loop or melting curve. (This may take a several seconds)
Are you sure that you want to stop the run in progress?	User pressed the stop button during a protocol.	User may either stop the protocol or choose not to stop.
There appears to be a run with no copy of Opticon Monitor watching it. Would you like to monitor this run?	Opticon Monitor detected a run-in-progress when it was launched.	User is given the opportunity to connect to this run and monitor it or open a copy of Opticon Monitor that does not monitor this run.
Do you want to save the changes you made to <FILENAME>	User is opening/closing a data file or creating a new master file. Opticon Monitor detected changes to the open file.	User may either select the option to save changes or not.

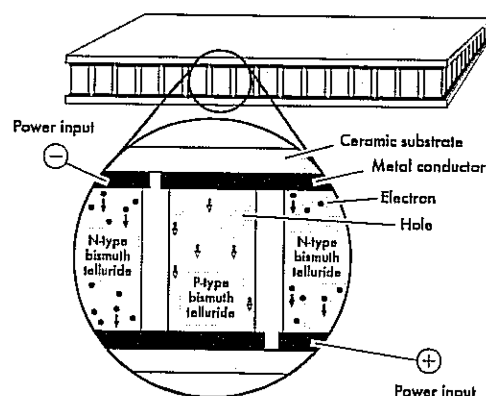
Appendix A

How a Peltier Heat Pump Works

The functional heart of every DNA Engine® thermal cycler is a high-performance Peltier-effect heat pump (also known as a "thermoelectric module"). The "MJ" module version is a solid-state device manufactured to withstand the thermal stresses associated with rapidly cycling temperatures.

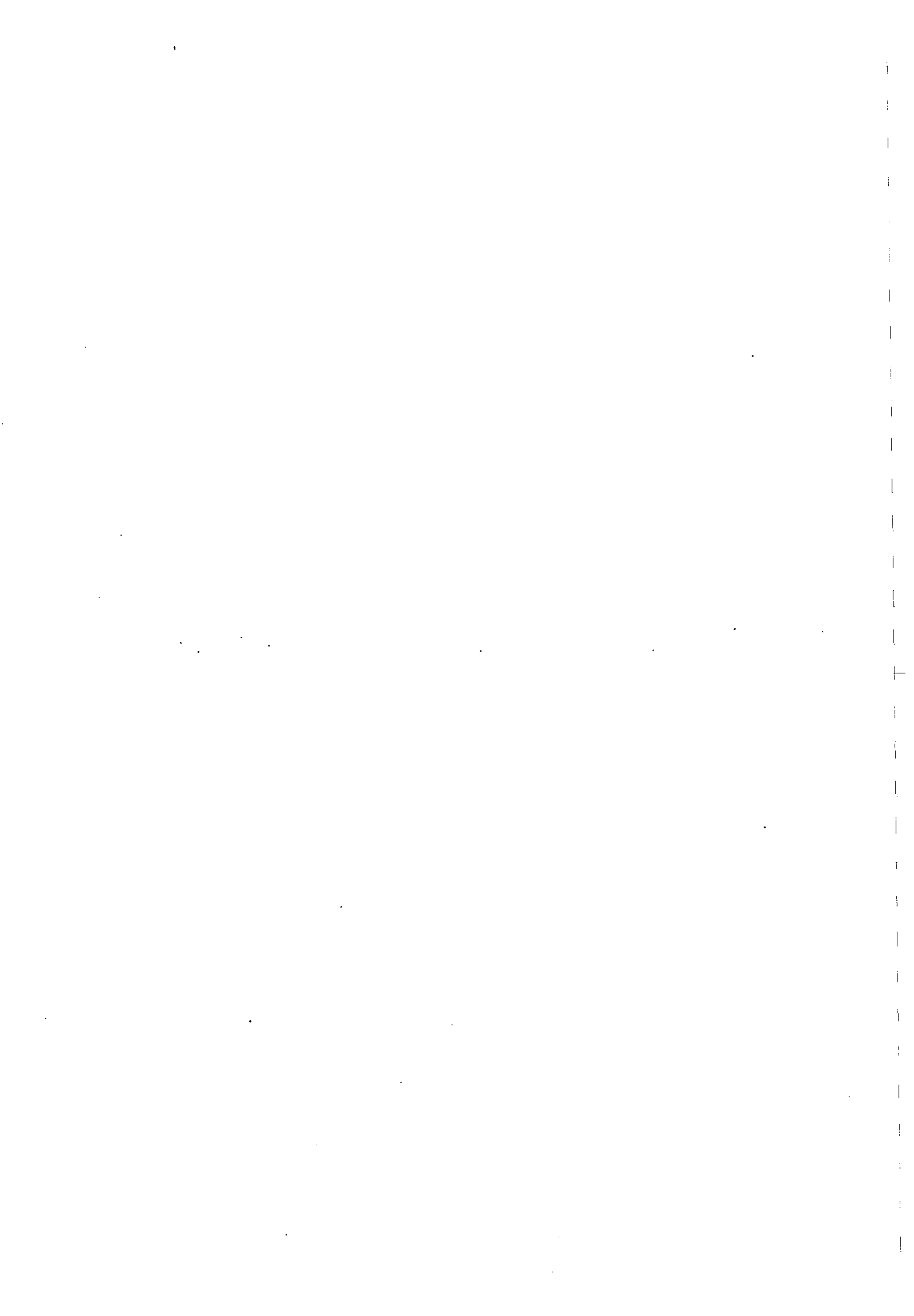
A thermoelectric module consists of numerous pairs of crystalline semiconductor blocks precisely sandwiched between two layers of ceramic substrate (fig. A-1). The blocks are of two varieties: "N-type," which has a surplus of electrons in its crystalline structure, and "P-type," which has a deficit of electrons. The two types are positioned in alternating pairs within the innermost layer of the sandwich.

Figure A-1 A thermoelectric module.



The two types of blocks are wired together in alternating pairs. When electrical current is passed through the blocks, electrons in the N-type blocks and the "holes," or empty electron spaces, in the P-type blocks are excited at one conductor–semiconductor interface, which absorbs a small amount of heat. The electrons and holes flow through the crystalline blocks and return to a low-energy state at the other conductor–semiconductor interface, with the release of the previously absorbed heat. A thermal gradient of up to 70°C can be generated across the blocks in this manner.

The direction of heat pumping is reversed by reversing the polarity of current flow through the thermoelectric module, and the amount of heat pumped is changed by changing the amount of current passed. Both direction and amount of current flow are dictated by a microprocessor, allowing precise control of thermal cycling in the Alpha™ unit block.

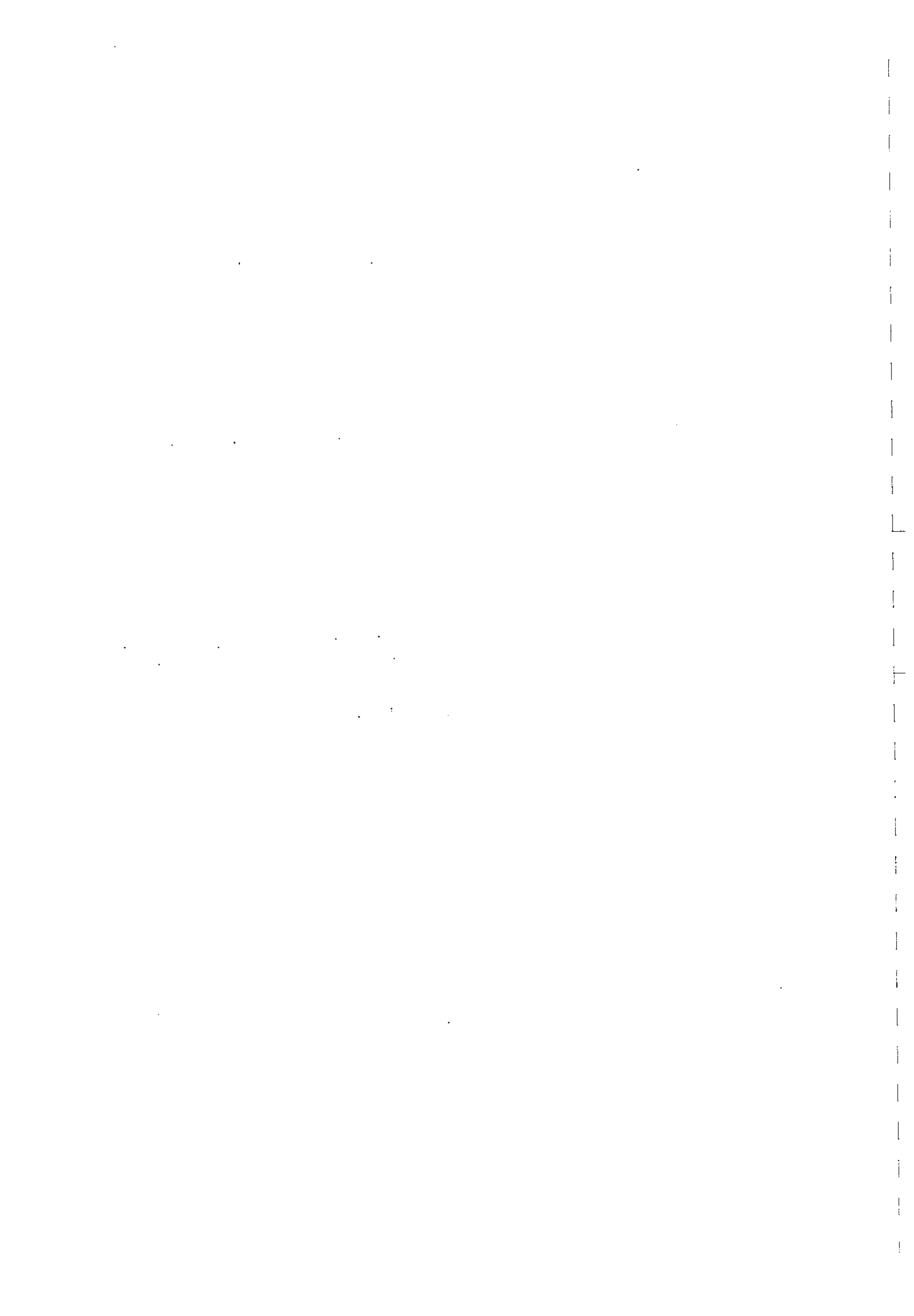


Appendix B

How a Switching Power Supply Works

Almost all solid-state electronic devices, including the DNA Engine® thermoelectric modules, require direct current (DC) for operation. However, electric utilities supply low-Hertz alternating current (AC), which varies in voltage and frequency from nation to nation. The Chromo4 system uses switching transistors, combined with high-frequency, resonant transformers, to convert the incoming AC to DC.

The power supply first chops the AC power into small bursts of energy (over 100,000 per second) with the aid of high-current switching transistors called MOSFETs (metal-oxide semiconductor field-effect transistors). The energy bursts are channeled into a high-frequency transformer. By changing the duration of the bursts that charge the transformer's magnetic core (pulse-width modulation), a specific voltage output can be maintained even when the incoming voltage varies (between 100 and 240 volts in the case of the Chromo4 system). Because the incoming power is being chopped so rapidly, the incoming frequency is unimportant; it can even be DC. Spikes and surges in the incoming power no longer pose a problem since they are chopped nearly to oblivion. The addition of resonance to the transformer design gives it extraordinary efficiency. These design innovations have made the Chromo4 system's power supply small in size, universal in input, and resistant to noise.



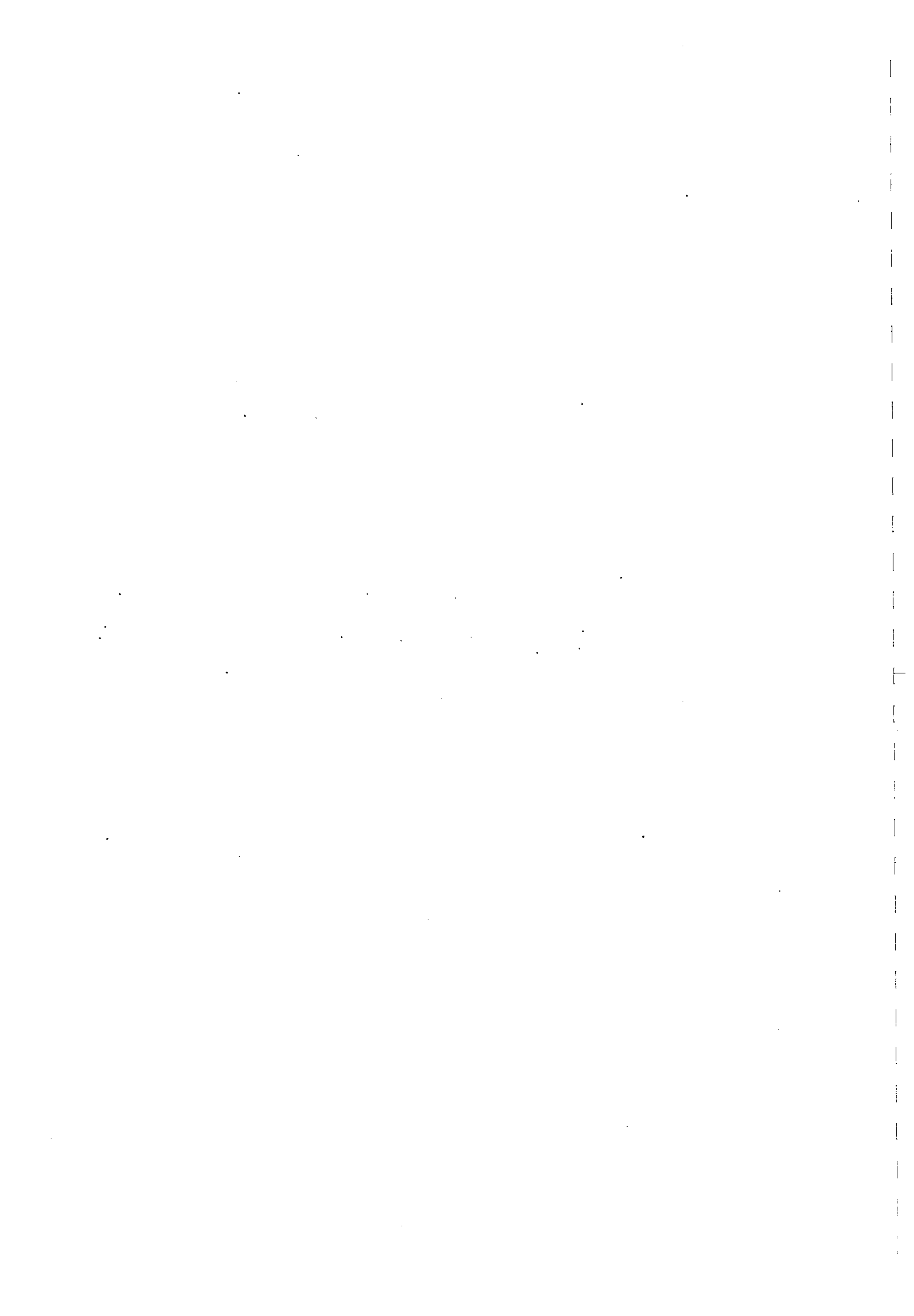
Appendix C

Shipping Instructions for US Residents

Users residing in the United States should follow these instructions for shipping a machine to MJ Research for factory repair or an upgrade. Users outside of the United States should send machines to their distributor, in accordance with shipping instructions obtained from the distributor.

1. Call MJ Research (888-652-9253) to obtain a return materials authorization (RMA) number. Machines returned without an RMA will be refused by the Receiving Department.
2. Thoroughly clean the machine, removing excess oil and radioactive and other biohazardous substances. To protect the health of our employees, MJ Research will not repair or upgrade any machine that is excessively oily or that emits ionizing radiation upon arrival at our factory. **PLEASE ELIMINATE ALL BIOHAZARDS AND RADIATION!**
3. Pack the machine in its original packaging. If this has been misplaced or discarded, call MJ Research to request shipment of packaging materials.
4. Write the RMA number on the outside of the box.
5. Ship the machine (freight prepaid) to the following address. We recommend you purchase insurance from your shipper.

   Ship to: Repair Department
   MJ Research, Incorporated
   590 Lincoln Street
   Waltham, MA 02451



Appendix D

Warranties

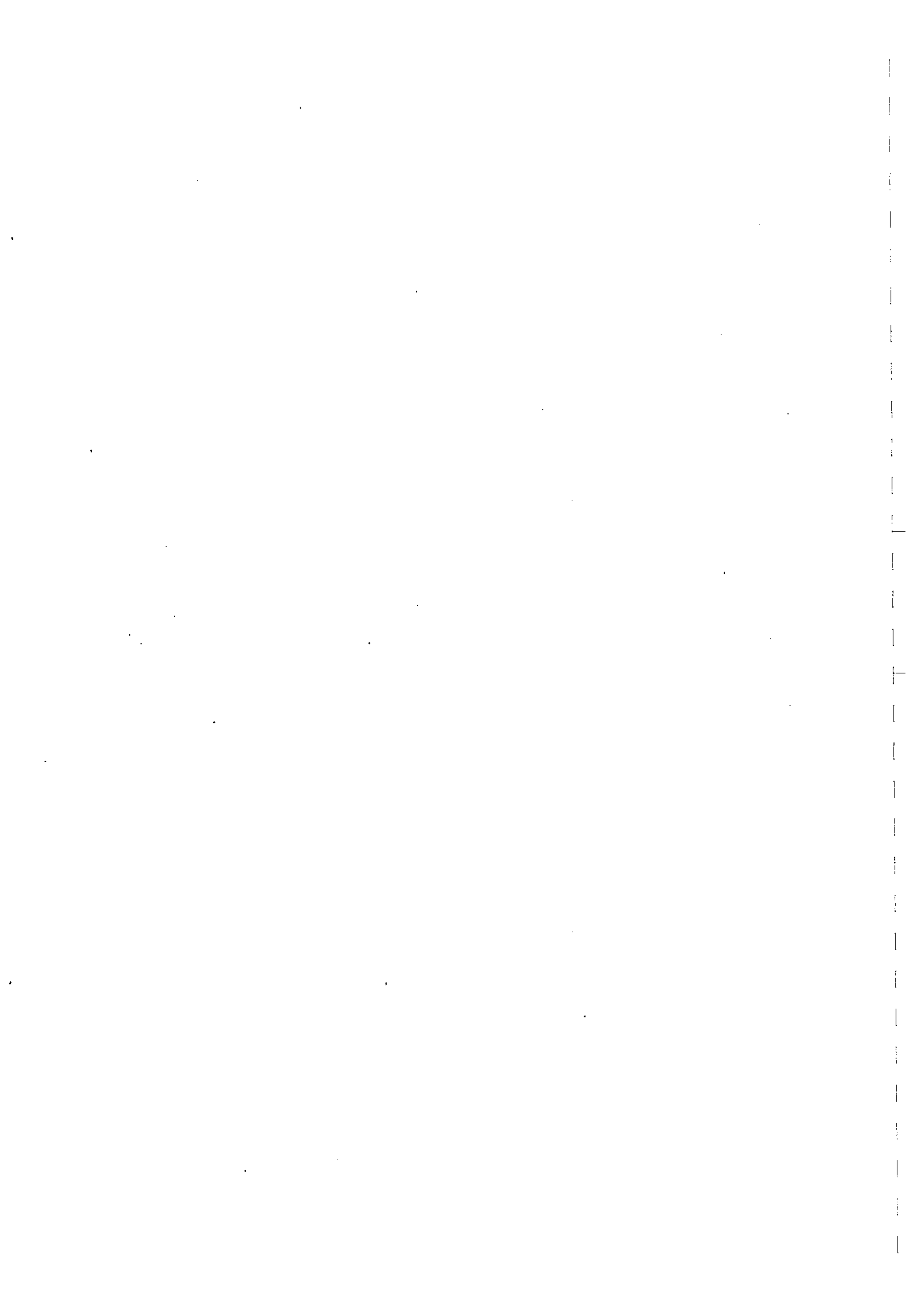
The Chromo4™ detector (CFD 3240 & ALS-3296) is warranted against defects in materials and workmanship. For specific Warranty information, contact your local Bio-Rad office. If any defects should occur during the warranty period, Bio-Rad will replace the defective parts without charge. However, the following defects are specifically excluded:

1. Defects caused by improper operation or by improper packaging of returned goods.
2. Repair or modifications done by anyone other than Bio-Rad Laboratories.
3. Use with tubes, plates, or sealing materials not specified by Bio-Rad Laboratories for use with the Chromo4 system.
4. Deliberate or accidental misuse.
5. Damage caused by disaster.
6. Damage due to use of improper solvent or sample.

The warranty does not apply to fuses.

For inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument.

For Technical Service call your local distributor or in the U.S. call 1-888-652-9253, or visit our website at www.mjr.com.



Appendix E

END-USER AGREEMENT AND LICENSE

FOR

MJ RESEARCH OPTICON MONITOR™ SOFTWARE

(ALL VERSIONS)

IMPORTANT! PLEASE READ THE FOLLOWING LICENSE AGREEMENT.

YOU HAVE PURCHASED AN MJ RESEARCH® CHROMO4™ CONTINUOUS FLUORESCENCE DETECTION SYSTEM THAT INCLUDES CERTAIN EQUIPMENT, DEVICES AND INSTRUMENTATION AS WELL AS A LICENSE TO COMPUTER SOFTWARE.

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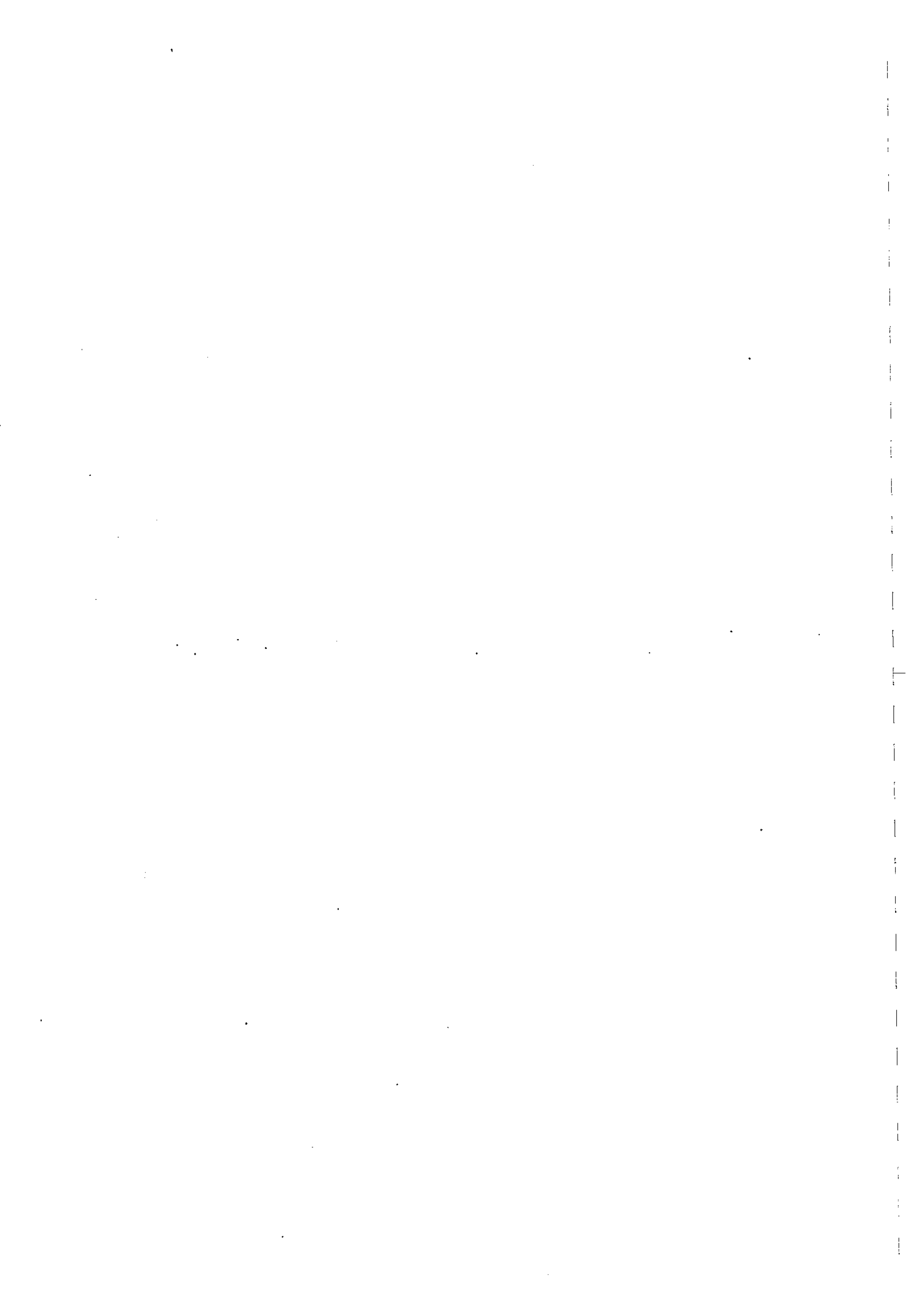

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Declaration of Conformity

Bio-Rad Laboratories, Inc., 1000 Alfred Nobel Drive, Hercules, California, 94547, U.S.A., declares that the product

CFD-3240, The Chromo4ⁱ Detection System

to which this declaration relates, is in conformity to the following standards or normative documents.

IEC61010-1
EN61326: CLASS A

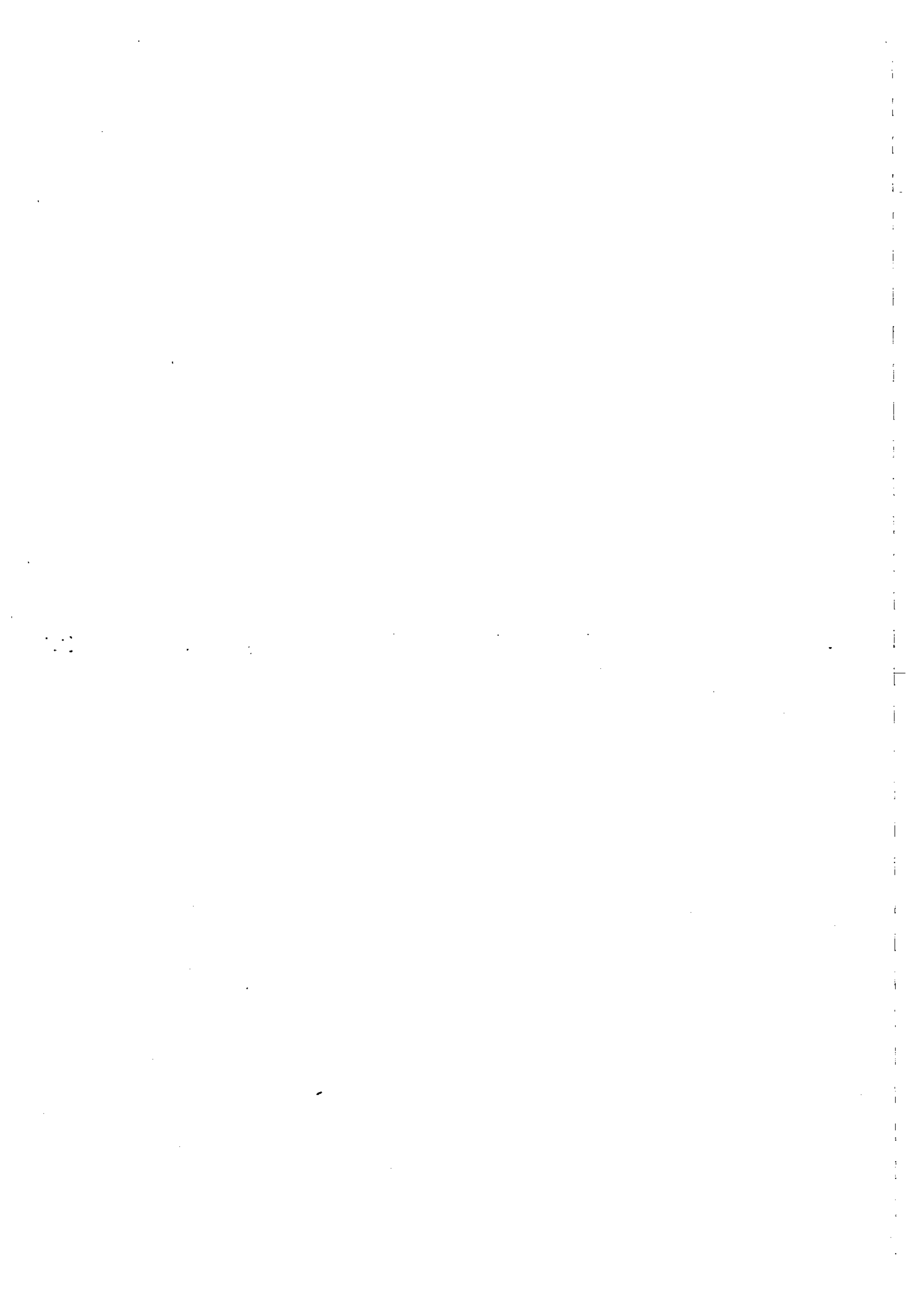
following the provisions of the 73/23/EEC, 89/336/EEC & 93/68/EEC Directive.

Test Data to verify this conformity are available for inspection at our European Representative Office at Literbuen 10B, 2740 Skovlunde, Denmark.

13 September, 2004
date of issue

Brad Crutchfield
Vice President

11149 rev A.A



DNA Engine® & DNA Engine Tetrad® Peltier Thermal Cyclers

Operations Manual

Version 4.0

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05434 revC.A DRAFT

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Applied Biosystems does not guarantee the performance of this instrument.

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Explanation of Symbols

CAUTION: Risk of Danger! Wherever this symbol appears, always consult note in this manual for further information before proceeding. This symbol identifies components that pose a risk of personal injury or damage to the instrument if improperly handled.

CAUTION: Risk of Electrical Shock! This symbol identifies components that pose a risk of electrical shock if improperly handled.

CAUTION: Hot Surface! This symbol identifies components that pose a risk of personal injury due to excessive heat if improperly handled.

Safety Warnings

Warning: Operating the DNA Engine® or DNA Engine Tetrad® cycler before reading this manual can constitute a personal injury hazard. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate these machines.

Warning: Do not open or attempt to repair the DNA Engine or DNA Engine Tetrad base, the DNA Engine Tetrad power supply, any Alpha™ unit, or other DNA Engine or DNA Engine Tetrad accessory. Doing so will void your warranties and can put you at risk for electrical shock. Return the DNA Engine or DNA Engine Tetrad instrument to the factory (US customers) or an authorized distributor (all other customers) if repairs are needed.

Warning: All Alpha unit blocks can become hot enough during the course of normal operation to cause burns or cause liquids to boil explosively. Wear safety goggles or other eye protection at all times during operation.

Warning: The DNA Engine and DNA Engine Tetrad instruments incorporate neutral fusing, which means that live power may still be available inside the machines even when a fuse has blown or been removed. Never open the DNA Engine or DNA Engine Tetrad base; you could receive a serious electrical shock. Opening the base will also void your warranties.

Caution: Never remove an Alpha unit from the DNA Engine or DNA Engine Tetrad base with the power turned on and a program running. Doing so can cause electrical arcing that can melt the contacts in the connector joining the Alpha unit to the DNA Engine or DNA Engine Tetrad base.

Safe Use Guidelines

The DNA Engine and DNA Engine Tetrad instruments are designed to be safe to operate under the following conditions:

- Indoor use
- Altitude up to 4000m
- Ambient temperature 5°–31°C
- Maximum relative humidity 90%, noncondensing
- Transient overvoltage per Installation Category II, IEC 664
- Pollution degree 2, in accordance with IEC 664

Electromagnetic Interference

These devices comply with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) these devices may not cause harmful interference, and (2) these devices must accept any interference received, including interference that may cause undesired operation.

These devices have been tested and found to comply with the EMC standards for emissions and susceptibility established by the European Union at time of manufacture.

This digital apparatus does not exceed the Class A limits for radio noise emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

LE PRESENT APPAREIL NUMERIQUE N'EMET PAS DE BRUITS RADIOELECTRIQUES DEPASSANT LES LIMITES APPLICABLES AUX APPAREILS NUMERIQUES DE CLASS A PRESCRITES DANS LE REGLEMENT SUR LE BROUILLAGE RADIOELECTRIQUE EDICTE PAR LE MINISTERE DES COMMUNICATIONS DU CANADA.

FCC Warning

Warning: Changes or modifications to these units not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radiofrequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Documentation Conventions

Typographic Conventions

The names of keyboard keys are set in sans serif type and encased in double angle brackets:

Example «Proceed»

Items in programming menus are italicized:

Example Select *Edit* from the Main Menu.

Graphic Conventions

The programming screens displayed in the LCD window are represented by a box containing four lines of text:

Example

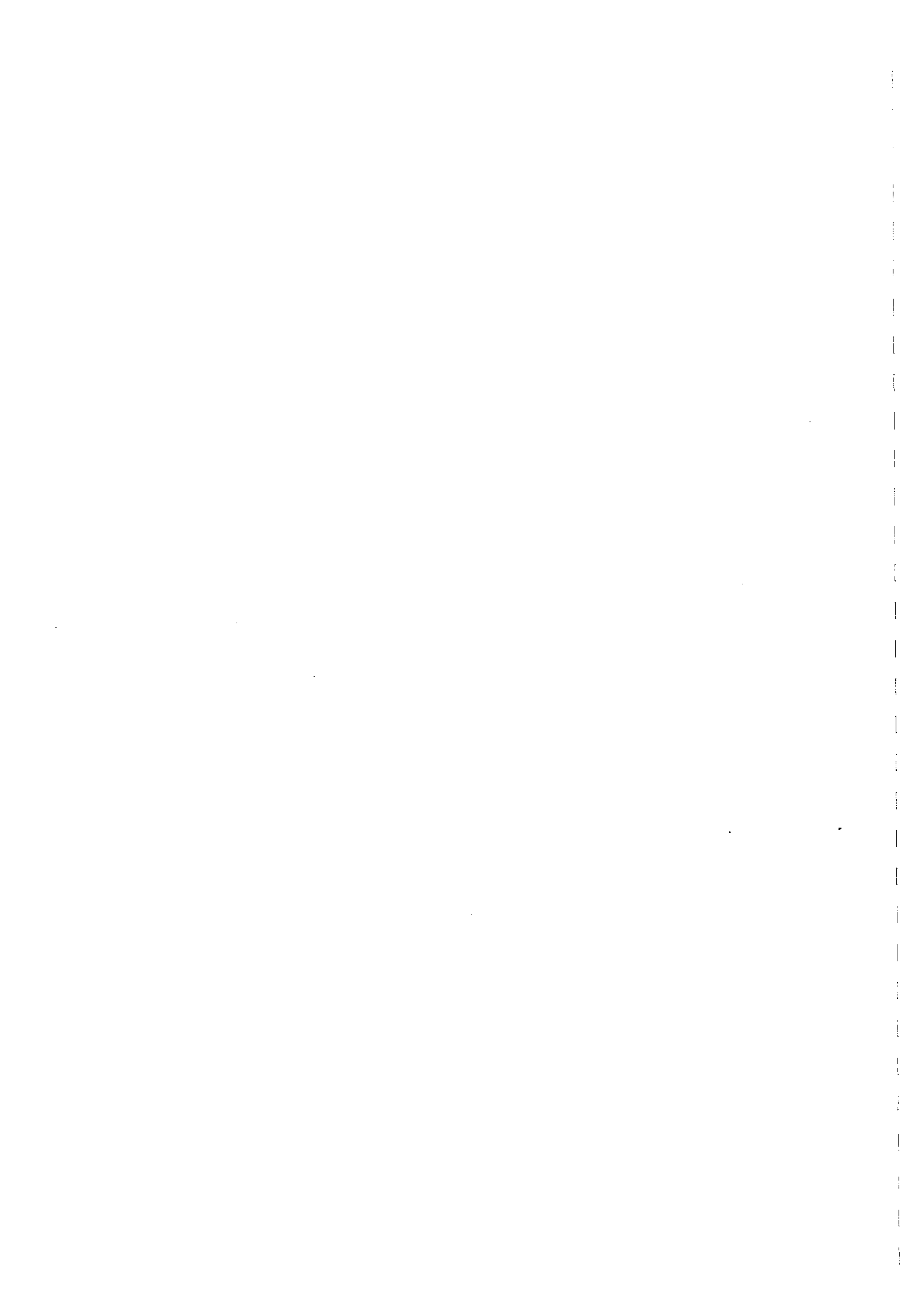
```
Run: 2-STEP
 1=  92.0  for 0:05
Cycle:  1
Calc:  65.0
```

Terminology

A programming option is termed "selected" when the cursor is positioned in front of it. Use the «Select» keys (see fig. 2-2) to move the cursor. In some screens selected items are also displayed in all-capital letters.

Part I

The DNA Engine® Peltier Thermal Cycler



Introduction

Meet the DNA Engine Thermal Cycler, *1-2*
Using This Manual, *1-2*
Important Safety Information, *1-3*

Meet the DNA Engine® Thermal Cycler

Thank you for purchasing an MJ RESEARCH® PTC-200 DNA Engine thermal cycler. Designed by a team of molecular biologists and engineers, the DNA Engine will meet your needs for a versatile, easy-to-use, reliable, and compact programmable thermal cycler:

- Interchangeable sample blocks—the Alpha™ unit family— that accommodate many types of tubes, microplates, and slides
- Hot Bonnet® heated lid (or its remote-controlled version, the Power Bonnet) for oil-free cycling
- Intuitive software with easy-to-read interface for quick and painless programming, editing, file management, password protection, and much more
- Choice of calculated sample temperature control for highest speed and accuracy, or block or probe control for compatibility with protocols designed for a variety of instrument types
- Space-saving design for easy setup and transportation
- Instant Incubate feature for continuous-temperature incubations
- Networking for up to 15 machines, for convenient remote operation and documentation of runs
- Customizable factory-installed protocols

When multiple users or high-throughput operations must be accommodated, the PTC-225 DNA Engine Tetrad® cycler is available. Essentially consisting of four DNA Engines placed in one chassis, the DNA Engine Tetrad has the same features and ease-of-use of the stand-alone DNA Engine.

Using This Manual

This manual contains all the information you need to operate your DNA Engine or DNA Engine Tetrad safely and productively:

- Chapter 2 acquaints you with the **physical characteristics** of the DNA Engine.
- Chapters 3–5 present the basics of **installing and operating** the DNA Engine.
- Chapters 6 and 7 describe **programming** the DNA Engine.
- Chapter 8 outlines the **utilities** available for the DNA Engine.
- Chapter 9 describes how to network **and remotely operate** the DNA

Engine and DNA Engine Tetrad.

- Chapter 10 explains the proper **maintenance** of the DNA Engine.
- Chapter 11 offers **troubleshooting** information for the DNA Engine.
- Chapter 12 describes the **operation of the DNA Engine Tetrad.**
- Chapter 13 describes the **operation of the Remote Alpha Dock™** accessory.

Important Safety Information

Safe operation of the DNA Engine or DNA Engine Tetrad begins with a complete understanding of how the machine works. Please read this entire manual before attempting to operate the DNA Engine or DNA Engine Tetrad. Do not allow anyone who has not read this manual to operate the machine.

The DNA Engine and DNA Engine Tetrad can generate enough heat to inflict serious burns and can deliver strong electrical shocks if not used according to the instructions in this manual. Please read the safety warnings and guidelines at the front of this manual, and exercise all precautions outlined in them.

2 Layout and Specifications

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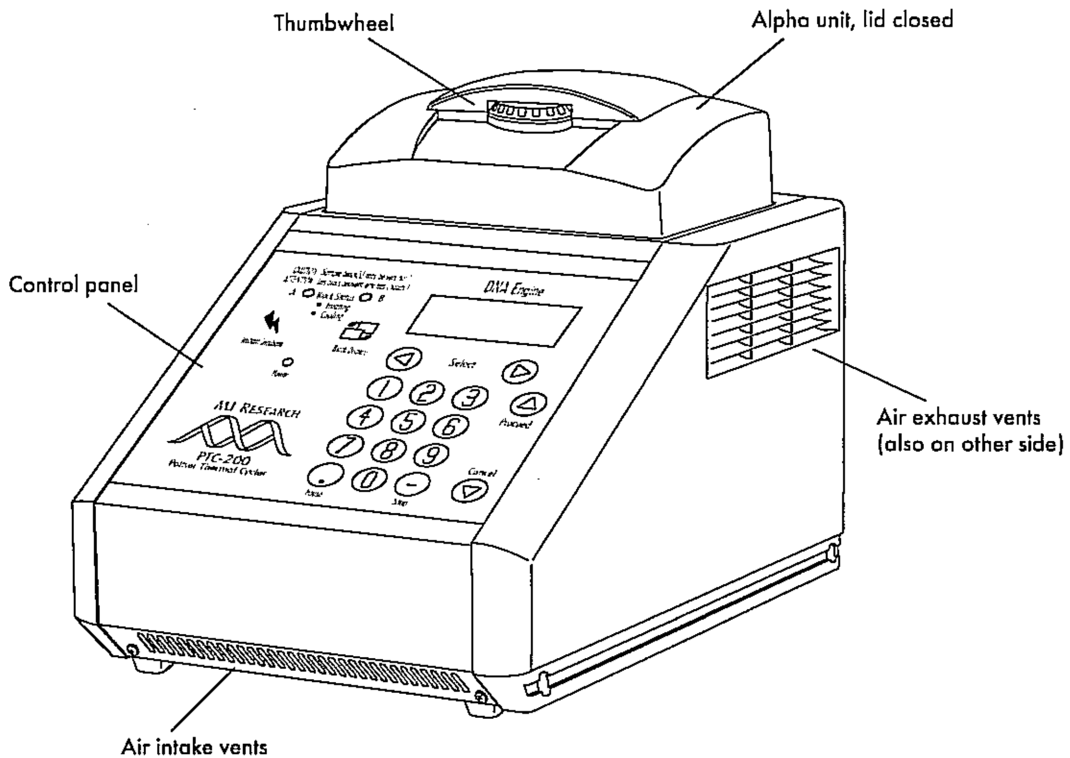
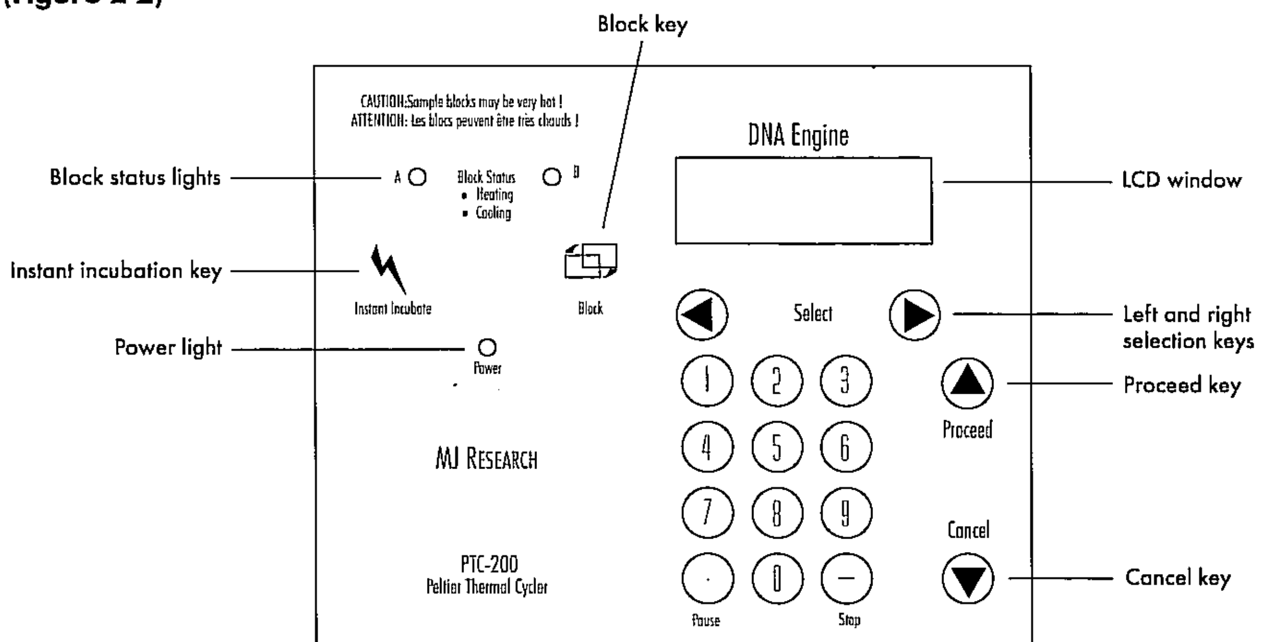
Front View

(Figure 2-1)



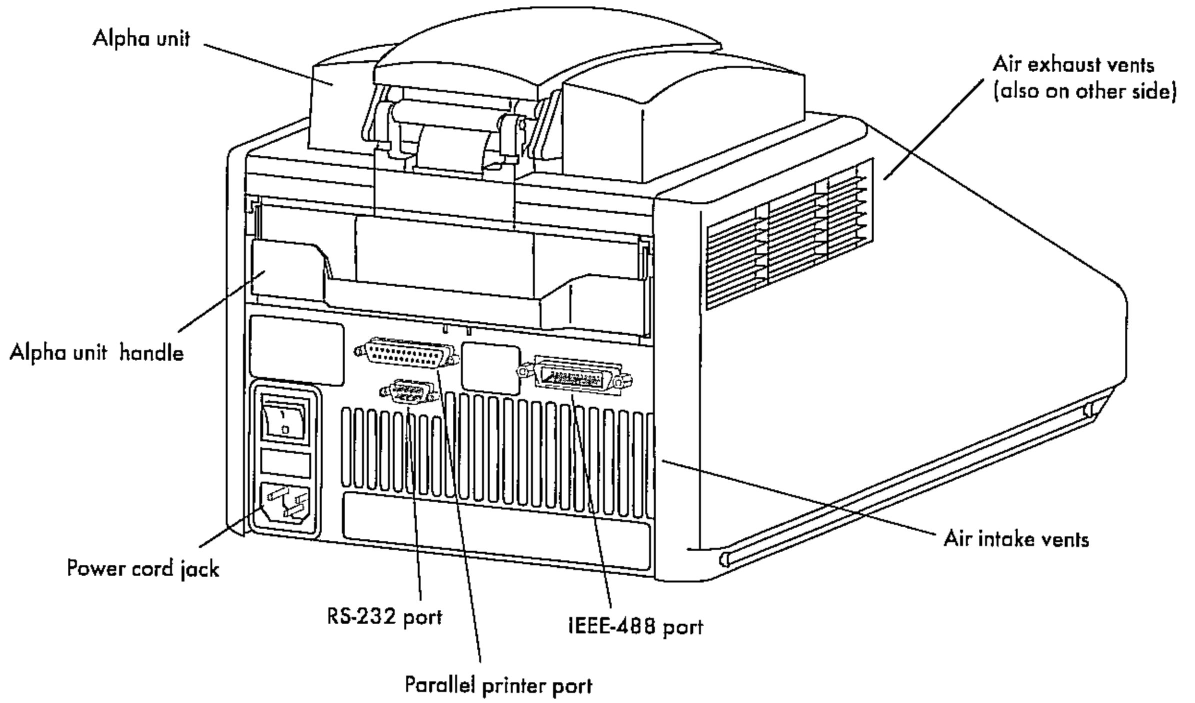
Control Panel

(Figure 2-2)



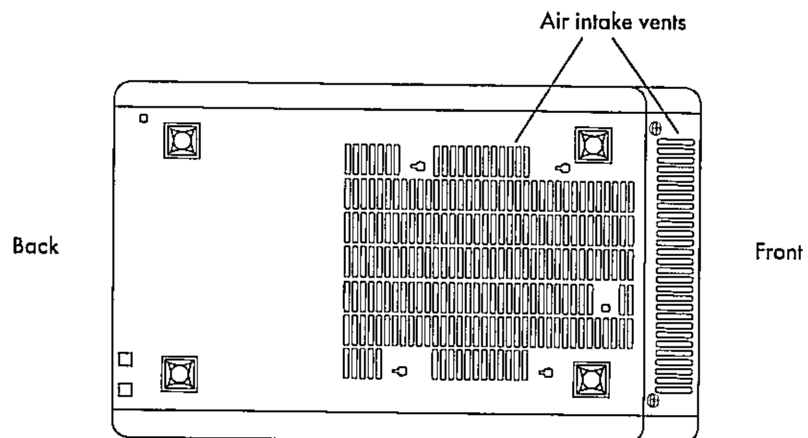
Back View

(Figure 2-3)



Bottom View

(Figure 2-4)



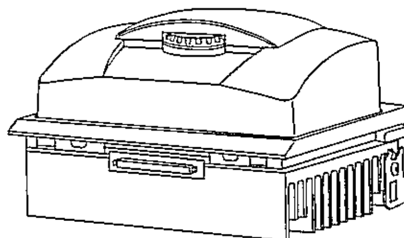
Alpha Units

Single-Block Models

60V Alpha: Holds 60 x 0.5ml tubes

96V Alpha: Holds 96 x 0.2ml tubes or one 96-well microplate

384 Alpha: Holds one 384-well microplate or one 96-well microplate

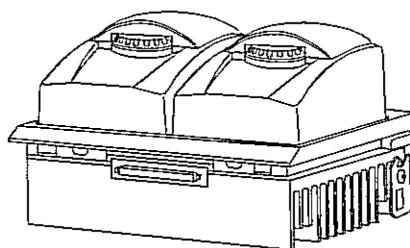


Dual-Block Models

30/30 Dual Alpha: Holds 2 x 30 x 0.5ml tubes

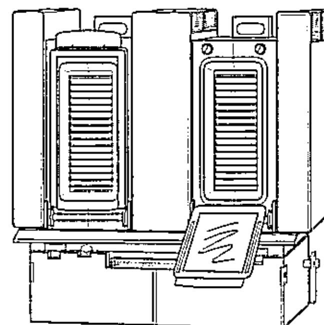
30/48 Dual Alpha: Holds 1 x 30 x 0.5ml tubes and 1 x 48 x 0.2ml tubes

48/48 Dual Alpha: Holds 2 x 48 x 0.2ml tubes or half plates



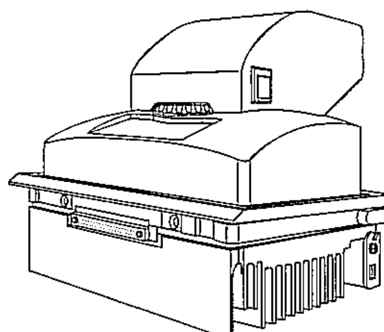
Slide Block

Slide Chambers Alpha unit: Holds 2 x 16 standard slides



Moto Alpha™ unit

Permits remote control of Alpha unit lid opening; available in 96-, 384-well and flat-block formats.



Specifications

Thermal range:	–5° to 105°C, but no more than 30°C below ambient temperature
Accuracy:	±0.3°C of programmed target @ 90°C, NIST-traceable
Thermal homogeneity:	±0.4°C well-to-well within 30 seconds of arrival at 90°C (for most Alpha units; see specifications for individual Alpha units)
Ramping speed:	Up to 3°C/sec for all single- and dual-block Alpha units; 1.2°C/sec for the Slide Chambers Alpha unit.
Sample capacity:	Varies with installed Alpha unit
Line voltage:	100–240VAC rms (no adjustment needed among voltages within these ranges)
Frequency:	50–60Hz single phase
Power:	850W maximum
Fuses:	Two 6.3A, 250V, 5 x 20mm
Displays:	One 20 x 4 LCD alphanumeric display
Ports:	One 25-pin 8-bit parallel interface printer port
	One 9-pin RS-232 serial port for printer or remote use
	One IEEE-488 bidirectional general purpose interface bus
Memory:	200 typical programs in up to 12 individual folders
Weight:	7.6–9.3kg, varies with installed Alpha unit (DNA Engine Tetrad: 29kg, including Alpha units and power supply)
Size:	24 x 35 x 17cm (DNA Engine Tetrad: 37 x 55 x 25cm)

Gradient Specifications (96V Alpha module only)

Accuracy:	± 0.4°C of programmed target at end columns, 30 seconds after the timer starts for the gradient step, NIST–traceable
Column uniformity:	± 0.4°C , well–to–well within column, within 30 seconds of reaching target temperature
Calculator accuracy:	± 0.4°C of actual well temperature
Lowest programmable temperature	30°C
Highest programmable temperature	105°C
Temperature differential range	1–24°C

3 Installation

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Setting Up the DNA Engine, *3-2*
Environmental Requirements, *3-2*
Power Supply Requirements, *3-3*
Air Supply Requirements, *3-3*
    Ensuring an Adequate Air Supply, *3-3*
    Ensuring That Air Is Cool Enough, *3-4*
Requirements for Robotics Installations, *3-5*

Packing Checklist

After unpacking the DNA Engine, check to see that you have received the following:

- One DNA Engine base
- One Alpha unit (more if additional units were ordered)
- Two spare fuses
- One power cord
- One probe, if purchased
- *PTC-200 DNA Engine & PTC-225 DNA Engine Tetrad Operations Manual* (this document)

If any of these components are missing or damaged, contact MJ Research or the authorized distributor from whom you purchased the DNA Engine to obtain a replacement. Please save the original packing materials in case you need to return the DNA Engine for service. See appendix C for shipping instructions.

Setting Up the DNA Engine

The DNA Engine requires only minimal assembly: plugging in the power cord and inserting an Alpha unit. Insert the power cord plug into its jack at the back of the machine (see fig. 2-3 for location of jack), then plug the cord into an electrical outlet. With the machine turned off, insert an Alpha unit (see "Installing an Alpha Unit," chapter 4).

Caution: Do not insert or remove an Alpha unit with the DNA Engine turned on; electrical arcing can result. Read the safety warning at the front of this manual regarding electrical safety when inserting or removing an Alpha unit.

Environmental Requirements

Ensure that the area where the DNA Engine is installed meets the following conditions, for reasons of safety and performance:

- Nonexplosive environment
- Normal air pressure (altitude below 4000m)
- Ambient temperature 5°–31°C
- Relative humidity up to 90%
- Unobstructed access to air that is 31°C or cooler (see below)

- Protection from excessive heat and accidental spills (Do not place the DNA Engine near such heat sources as radiators, and protect it from danger of having water or other fluids splashed on it, which can cause shorting in its electrical circuits.)

Power Supply Requirements

The DNA Engine requires 100–240VAC, 50–60Hz, and a grounded outlet. The DNA Engine can use current in the specified range without adjustment, so there is no voltage-setting switch.

Power cords for outlets other than the US 120V outlet may be purchased from computer stores, since they are also used for most desktop computers and printers and meet international standard IEC-320. The power cord must be rated to carry at least 10A at 125V or 250V, depending on the voltage available in your nation. The quality of the power cord can be further ensured by making certain it is inscribed with the trademark of UL, CSA, TUV, VDE, or another national testing agency.

Note: Do not cut the supplied 120V power cord and attach a different connector. Use a one-piece molded connector of the type specified above.

Air Supply Requirements

The DNA Engine requires a constant supply of air that is 31°C or cooler in order to remove heat from the Alpha unit's heat sink. Air is taken in from vents at the front, back, and bottom of the machine and exhausted from vents on both sides (see figs. 2-1, 2-3, and 2-4). If the air supply is inadequate or too hot, the machine can overheat, causing performance problems, software error messages (particularly "HS Overheating" and "Slow Block Cycling"), and even automatic shutdowns. Special attention should be paid to airflow and air temperature in robotics installations of DNA Engines.

Ensuring an Adequate Air Supply

- Do not block the air intake vents.

  Position the DNA Engine at least 10cm from vertical surfaces and other thermal cyclers (greater distances may be required; see below). Do not put loose papers under the machine; they can be sucked into the air intake vents on the bottom of the machine.

- Do not allow dust or debris to collect in the air intake vents.

  The bottom air vents are particularly liable to collect dust and debris, sometimes completely clogging up. Check for dust and debris every

few months, and clean the intake vents as needed. Remove light collections of dust with a soft-bristle brush or damp cloth. Severe collections of dust and debris should be vacuumed out. Turn the machine off prior to cleaning or vacuuming air vents.

Ensuring That Air Is Cool Enough

- Do not position two or more DNA Engines (or other thermal cyclers) so that the hot exhaust air of one blows directly into the air intake vents of another.
- Make sure the DNA Engine receives air that is 31°C or cooler by measuring the temperature of air entering the machine through its air intake vents.

  Place the DNA Engine where you plan to use it, and turn it on. Try to reproduce what will be typical operating conditions for the machine in that location, particularly any heat-producing factors (e.g., nearby equipment running, window blinds open, lights on). Run a typical protocol (e.g., 2-Step) for 30 minutes to warm up the DNA Engine, then measure the air temperature at the back air intake vents. If more than one machine is involved, measure the air temperature for each.

  If the air intake temperature of any machine is warmer than 31°C, use table 3-1 to troubleshoot the problem. Some experimentation may be required to determine the best solution when more than one cause is involved. After taking steps to solve the problem, verify that the temperature of the air entering the air intake vents has been lowered, using the procedure outlined above.

Table 3-1 Troubleshooting Air Supply Problems

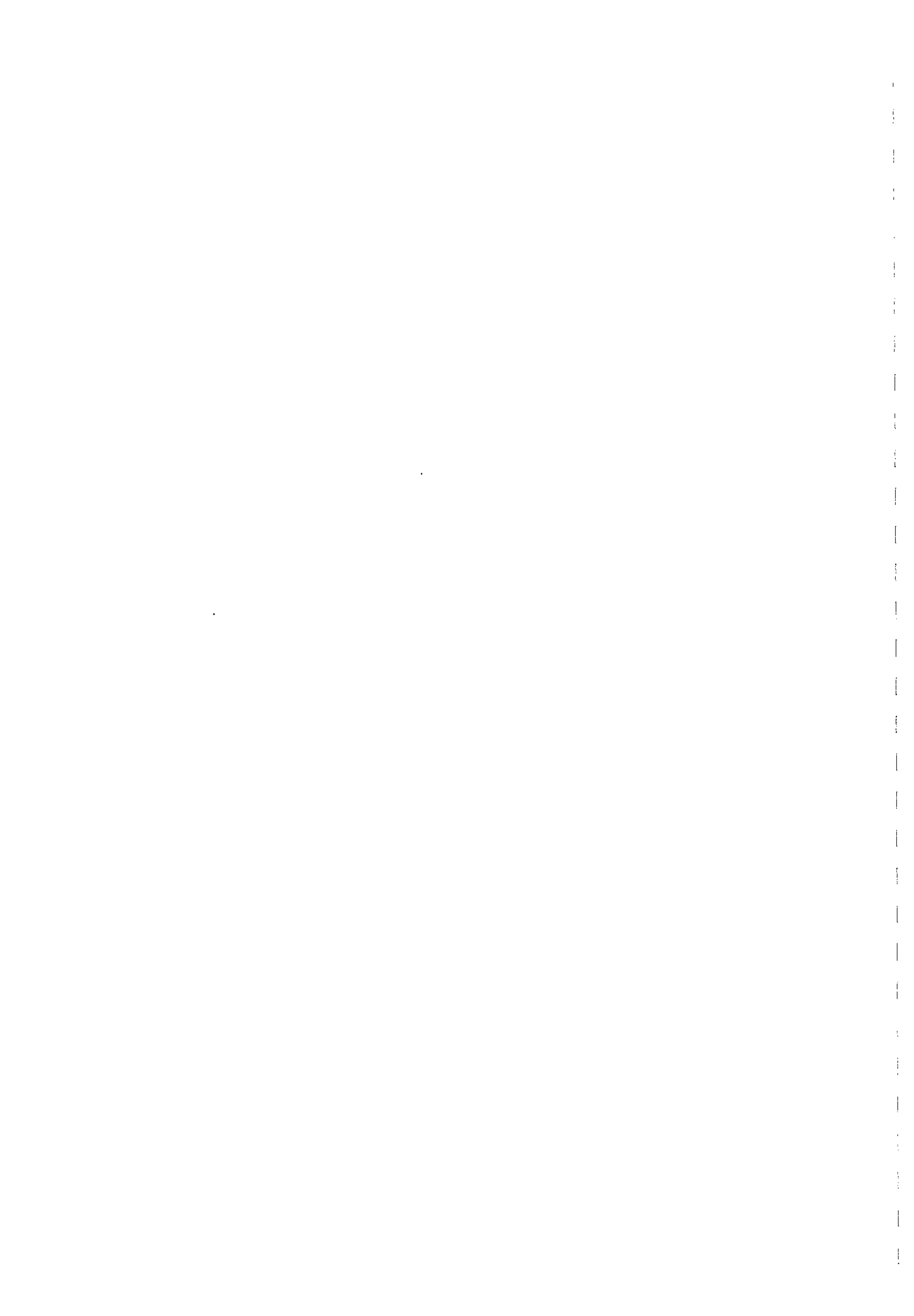
Cause	Possible Remedies
Air circulation is poor.	Provide more space around machine or adjust room ventilation.
Ambient air temperature is high.	Adjust air conditioning to lower ambient air temperature.
Machine is in warm part of room.	Move machine away from, or protect machine from, such heat sources as radiators, heaters, other equipment, or bright sunlight.
Machines are crowded.	Arrange machines so that warm exhaust air does not enter intake vents.

Requirements for Robotics Installations

Robotics installations require special attention to airflow and air temperature. Typically in these installations, DNA Engines and other thermal cyclers are crowded into a small area, along with other heat-generating equipment. Overheating can quickly occur when many of these machines are operating at once, unless preventive measures are taken.

Follow the procedures described above to ensure adequate airflow and an air intake temperature of 31°C or cooler. Air intake temperature must be verified by measurement.

Do not use oil to thermally couple sample vessels to the blocks of machines in a robotics installation. Oil makes plates difficult to remove.



4 Operation

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Understanding the Main Menu, *4-2*
Using the Control Panel, *4-3*
  Operation Keys, *4-3*
  Status Indicator Lights, *4-3*
Using the Data Ports, *4-3*
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Turning the DNA Engine On

Move the power switch to "1" (the "On" position). In most The fan will turn on, and the power light on the keyboard will glow red. In most cases a self-test of the heat pumps will begin running (see note below). Its progress is tracked on a screen in the LCD window:

```
PTC-200:

    Self testing
```

This screen disappears in 1 minute. If a problem is detected, the display shows an error message.

Note: If the Alpha unit's block or heat sink is not at ambient temperature (typically because the Alpha unit was recently in use), the machine will skip the self-test.

If the self-test does not detect any problems, the Main Menu is displayed:

```
PTC-200:
_RUN       Enter
 List      Edit
 Files     Setup
```

The DNA Engine is now ready to execute programs.

Understanding the Main Menu

The Main Menu is the common access point to all programming and machine configuration screens:

- **Run:** Executes a program.
- **Enter:** Allows new programs to be entered.
- **List:** Accesses utilities that display or print a program's steps.
- **Edit:** Allows modification of stored programs.
- **Files:** Accesses file management utilities.
- **Setup:** Accesses machine and networking configuration screens.

Using the Control Panel

The control panel (see fig. 2-2) includes operation keys, status indicator lights, an LCD window for displaying programming and machine status text, and a numeric keypad for entering values into programs.

Operation Keys

- **Select keys** (left and right arrows): Move the cursor one space or option to the left or right in the LCD window; display time and cycle information during a protocol run.
- **Proceed:** Accepts a selected menu or screen option; during a protocol run, advances the program to its next step.
- **Cancel:** Terminates a running protocol; during program creation or editing, cancels the last entry.
- **Stop:** Terminates a running protocol.
- **Pause:** Pauses a protocol during execution; accesses Japanese *Katakana* syllabary.
- **Instant Incubate:** Initiates a program that sets up the DNA Engine as a simple incubator.
- **Block:** Selects a different block when using a dual-block Alpha unit; switches between block screens and the Main Menu in the LCD window during a protocol run.

Status Indicator Lights

- **Power light:** Glows red when the DNA Engine is powered up.
- **Block Status lights:** Indicate which blocks are in use; glow red when blocks are heating and green when blocks are cooling.

Using the Data Ports

The DNA Engine has three data ports located at the rear of the machine: an RS-232 port, an IEEE-488 port, and a parallel (printer) port. See chapters 8 and 9 for information on using these ports to network machines, connect them to a computer, or print data.

Operating Alpha Units

Note: Operation of the Slide Chambers Alpha unit will not be discussed, owing to the many differences between this type of Alpha unit and the others. Please see the *Slide Chambers Operations Manual* for operating instructions.

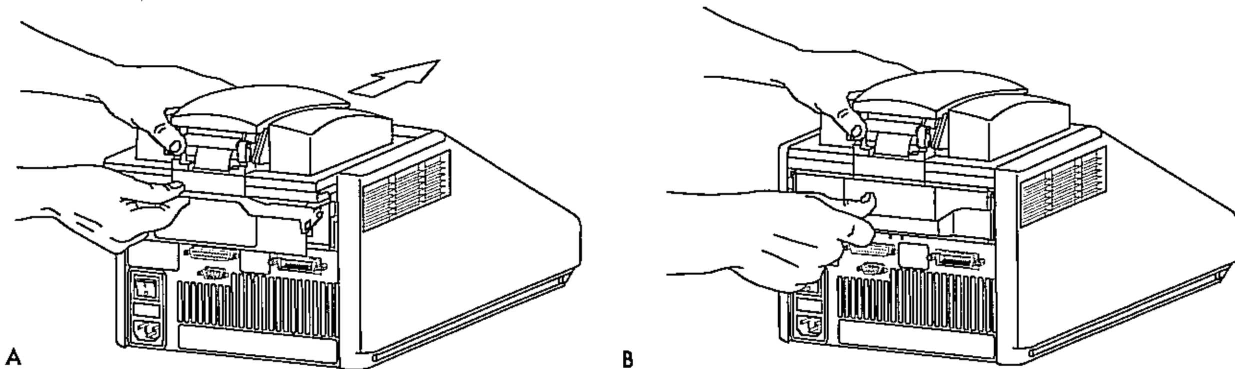
Note: Moto Alpha™ units are installed and removed as described below. See the *Moto Alpha Unit Operations Manual* for instructions on opening and closing Moto Alpha units.

Installing an Alpha Unit

1. **Turn the DNA Engine off (see the Caution on p. 4-5).**
2. Hold the Alpha unit at its front and back edges.
3. Lower the Alpha unit into the DNA Engine base, leaving at least 3cm between the front edge of the Alpha unit and the front of the base.
4. Raise the handle at the back of the Alpha unit, and slide the block forward as far as it will go (fig. 4-1A).
5. Push the handle down until it is completely vertical (fig. 4-1B); firm pressure may be required. A definite click signals that the Alpha unit's connectors have mated with the DNA Engine's connectors.

When the handle is in the down position, the Alpha unit is locked into place.

Figure 4-1 Installing an Alpha unit.



Removing an Alpha Unit

1. **Turn the DNA Engine off (see the Caution below).**
2. Pull upward on the handle. When the lock releases, you will hear a click, and the Alpha unit will slide a little toward the back of the DNA Engine. The electrical connectors of the Alpha unit and the DNA Engine are now disengaged, so there is no danger of electrical shock.
3. Slide the Alpha unit toward the rear of the DNA Engine about 3cm.
4. Grasp the front and back edges of the Alpha unit, and lift it out of the machine.

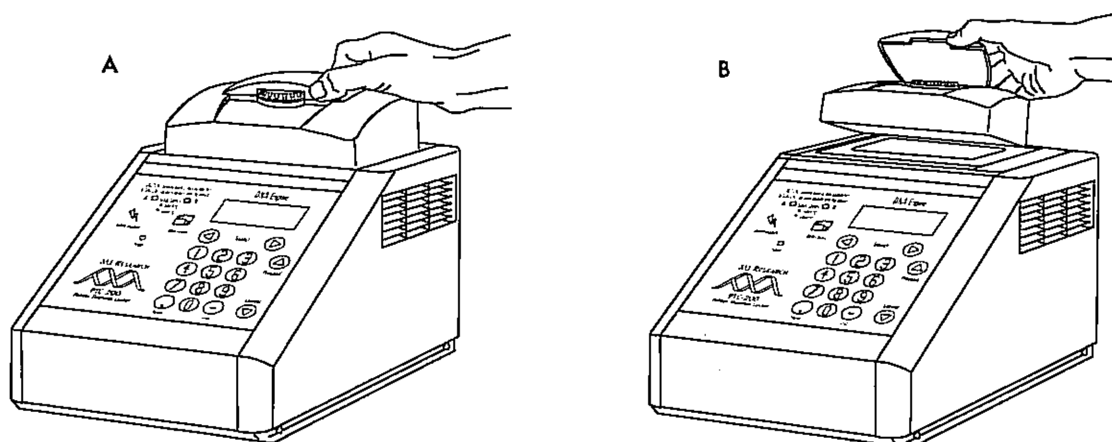
⚠ **Caution:** Do not insert or remove an Alpha unit with the DNA Engine turned on; electrical arcing can result. Read the safety warning at the front of this manual regarding electrical safety when inserting or removing an Alpha unit.

Opening an Alpha Unit

Grip the front edge of the top lever of the Hot Bonnet® as shown in figure 4-2A, and pull upward firmly. The top lever will pop open to reveal the entire thumbwheel (fig. 4-2B). Continue pulling upward to open the Hot Bonnet. The Hot Bonnet will tip backward, revealing the entire block.

⚠ **Caution:** Do not pull on the thumbwheel to open the unit. This can damage the Hot Bonnet's closing mechanism.

Figure 4-2 Opening an Alpha unit.



Closing an Alpha Unit

Press down on the top lever. The lever will close down over the thumbwheel as the Hot Bonnet closes down over the sample block. A click signifies that the Hot Bonnet's latch has engaged.

Selecting the Correct Sample Vessel

The DNA Engine's wide variety of interchangeable Alpha units affords you great scope in choosing sample vessels. Keep in mind that differences in tube and plate composition and wall thickness among the many brands available can affect reaction performance. Protocols may require some adjustment to ensure optimum performance when using a new vessel type. MJ RESEARCH offers a full range of tubes and microplates, manufactured to the specifications of each type of Alpha unit to ensure a precise fit. See chapter appendix 4-A for a complete list.

0.5-ml Tubes

Make sure thick-walled 0.5-ml tubes fit the wells snugly. Since these tubes were originally designed for centrifuges, some brands may not fit tightly in thermal cycler wells. Thin-walled 0.5-ml tubes were specifically designed for thermal cycling, and the higher quality brands provide a good and consistent fit. MJ RESEARCH provides thin- and thick-walled 0.5-ml tubes designed for precise block fit.

0.2-ml Tubes

All types of thin-walled 0.2-ml tubes may be used. MJ RESEARCH sells high-quality 0.2ml tubes in a number of styles, including individual tubes and strips.

Microplates

A variety of 96-well polycarbonate or polypropylene microplates can be used in 96V Alpha units as long as they fit the wells snugly. Polypropylene microplates are usually preferred because they exhibit very low protein binding and, unlike polycarbonate microplates, do not lose water vapor through the vessel walls. This allows smaller sample volumes to be used—as little as 5–10µl. Polypropylene microplates and compatible Microseal® 'A' film, mats, or strip caps for sealing are available from MJ RESEARCH. (See "Sealing with the Hot Bonnet and Caps or Film Sheets," p. X, for a description of Microseal 'A'.)

Thin-Walled Vs. Thick-Walled Tubes

The thickness of sample tubes directly affects the speed of sample heating and thus the amount of time required for incubations. Thick-walled tubes delay sample heating, since heat transfers more slowly through the tubes' walls. For the earliest types of thermal cyclers this delay mattered little. These machines' ramping rates were so slow (below 1°C/sec) that there was plenty of time for heat to transfer through the tube wall to the sample, during a given incubation.

Modern thermal cyclers have much faster ramping rates (up to 2–3°C/second), so the faster heat transfer provided by thin-walled tubes allows protocols to be significantly shortened. For example, in the reaction illustrated in figure 4-x, over 30 seconds can be saved per cycle by using thin-walled tubes, for an overall savings of 15 minutes in a 30-cycle run.

Sealing Sample Vessels

Steps must be taken to prevent the evaporation of water from reaction mixtures during thermal cycling, to avoid changing the concentration of reactants. Only a layer of oil or wax will completely prevent evaporation from sample vessels. However, an adequate degree of protection can be achieved by sealing with Microseal film or caps, then cycling the samples using the heated lid to prevent condensation/refluxing.

Sealing with Oil or Wax

Mineral oil, silicone oil, paraffin wax, or Chill-out™ liquid wax may be used to seal sample vessels. Use only a small amount of oil or wax; 1–3 drops (15–50µl) are usually sufficient. (Include this volume in the total volume when setting up a calculated-control protocol; see "Setting Up a Calculated-Control Protocol," chapter 5.) **Use the same amount of oil or wax in all sample vessels to ensure a uniform thermal profile.**

Most paraffin waxes solidify at room temperature. The wax can then be pierced with a micropipette and the samples drawn off from below the wax. Silicone oil and mineral oil can be poured off or aspirated from tubes if the samples are first frozen (–15° to –20°C). The samples are usually pure enough for analysis without an extraction.

Chill-out liquid wax (available from MJ Research) is an easy-to-use alternative to oil. This purified paraffinic oil solidifies at 14°C and is liquid at room temperature. By programming a hold at low temperature, the wax can be solidified at the end of a run. A pipette can then be used to pierce the wax in the tubes and remove the samples. The wax is dyed red to assist in monitoring its use. The dye has no adverse effects on fluorescent gel analysis of reaction products.

Sealing with the Hot Bonnet and Caps or Film Sheets

The Hot Bonnet's heated inner lid maintains the air in the upper part of sample vessels at a higher temperature than the reaction mixture. This prevents condensation of evaporated water vapor onto the vessel walls, so that solution concentrations are unchanged by thermal cycling. The Hot Bonnet also exerts pressure on the tops of vessels loaded into the block, helping to maintain a vapor-tight seal and to firmly seat tubes or the plate in the block.

Caps, film, or mats must be used along with the Hot Bonnet to prevent evaporative losses. Tight-fitting caps do the best job of preventing vapor loss (and should be used for long-term storage of reaction products).

Microseal 'A' film is a quick way to seal Multiplate and "Concord" microplates or large arrays of tubes. This film is specially designed to seal tightly during cycling yet release smoothly, which minimizes the risk of aerosol formation and cross-contamination of samples. Microseal 'A' is easily cut for use with fewer than 96 wells.

Microseal 'M' rubber sealing mats are an economical means to seal 96-well microplates. An array of 96 dimples on the mat helps orient it on the microplate and prevents the mat from sticking to the Hot Bonnet's heated lid. The mats may be cleaned with sodium hypochlorite for reuse and are autoclavable.

Note: After a hold at below-ambient temperatures, a ring of condensation may form in tubes above the liquid level but below the top of the sample block. This is not a cause for concern since it occurs only at the final cool-down step, when thermal cycling is finished.

Adjusting the Hot Bonnet's Lid Pressure

The pressure exerted by the inner lid of the Hot Bonnet must be manually adjusted to fit the sample vessels being used in a given reaction. Once set, the Hot Bonnet can be opened and closed repeatedly without readjustment as long as neither the tube or microplate type nor the sealing method changes. Any change in vessel type or sealing method requires readjustment of the Hot Bonnet's lid.

Follow these steps to adjust the pressure exerted by the Hot Bonnet's inner lid:

1. Make sure the block's wells are clean. Even tiny amounts of extraneous material can interfere with the proper seating of a microplate or tubes, which would prevent the inner lid from exerting uniform pressure on the loaded microplate or tubes.
2. Open the Hot Bonnet. Turn the blue thumbwheel all the way counterclockwise to completely raise the inner lid.

3. Load either a microplate or at least eight individual tubes into the sample block. The inner lid pivots around a central point, so it is important to distribute individual tubes evenly: Load at least four tubes in the center of the block and at least one tube in each of the four corners of the block. If using a sealing film or mat, apply it to the loaded microplate according to the manufacturer's directions.
4. Close the Hot Bonnet by pressing down on the top lever. Turn the thumbwheel clockwise to lower the Hot Bonnet's inner lid onto the loaded microplate/tubes. The thumbwheel turns easily at first since the inner lid has not yet come into contact with anything. Stop turning the thumbwheel when you feel increased resistance, which indicates that the inner lid has touched the microplate/tubes.
5. Open the Hot Bonnet. Turn the thumbwheel clockwise an extra half to three-quarters of a turn to set an appropriate lid pressure.

Caution: Do not turn the thumbwheel more than three-quarters of a turn. This can make it hard or impossible to close the lid and puts excessive strain on the latch holding the lid closed.

An extra half to three-quarters of a turn ensures the correct pressure for most types of reaction vessels. Some empirical testing may be required to determine the optimum pressure required for certain vessels. Once this pressure has been determined, the thumbwheel position that delivers it may be marked with a colored marking pen or piece of tape.

Note: As an aid in gauging how much the thumbwheel has been turned, mark it at the quarter turn positions, or every sixth "bump" on the thumbwheel (there are 24 total "bumps").

6. Close the Hot Bonnet.

Loading Sample Vessels into the Block

When using a small number of tubes, they should all be placed in the center of the block, to ensure uniform thermal cycling of all samples. Also load at least one empty tube in each corner of the block, to ensure that the Hot Bonnet exerts even pressure on the sample tubes (see "Adjusting the Hot Bonnet's Lid Pressure," p. 4-8).

To ensure uniform heating and cooling of samples, sample vessels must be in complete contact with the block. Adequate contact is ensured by always doing the following:

- Ensure that the block is clean before loading samples (see chapter 10 for cleaning instructions)
- Firmly press individual tubes or the microplate into the block wells.

Using Oil to Thermally Couple Sample Vessels to the Block

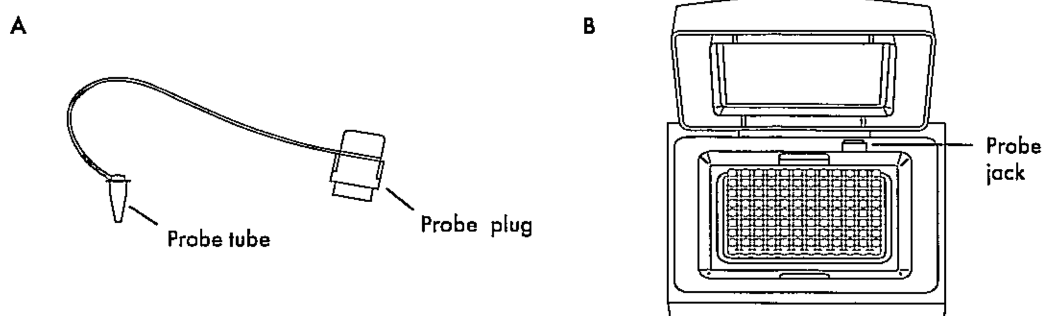
With two exceptions (see below), MJ RESEARCH does not recommend using oil to thermally couple sample vessels to the block, for the following reasons:

- Calculated-control protocols do not run accurately when oil is used.
- Oil traps dirt, which interferes with thermal contact between vessels and the block.

Caution: If you use oil in the block, use only mineral oil. **Never use silicone oil.** It can damage the Alpha unit.

One exception to this recommendation involves the use of volatile radioactive ^{35}S nucleotides. A small amount of oil in the block can help prevent escape of these compounds. See chapter appendix 4-B for important information regarding safe use of these compounds in polypropylene tubes and polypropylene and polycarbonate microplates. A second exception involves the use of thick-wall 0.5-ml tubes. Certain brands of these tubes fit poorly in the block, in which case oil may somewhat improve thermal contact. Whenever possible, use high-quality thin-wall tubes intended for thermal cycling (see chapter appendix 4-A for a tube and plate selection chart).

Figure 4-3 A, Probe. B, Location of probe jack.



Using the Optional Probe

The probe consists of a precision thermistor mounted in a thin-walled plastic tube. A thin wire, encased in a small plastic tube, runs from the thermistor to the probe's plug, which is inserted into a slot at the back of the Alpha unit (fig. 4-3). A small amount of oil is added to the probe tube to serve as the representative sample. The tube is loaded into the block, where it can serve as the control reference for any programmed target temperature between 0° and 100°C.

When a probe-control protocol is run, the DNA Engine controls block temperature to keep the probe vessel at the programmed temperature, using feedback information from the thermistor. (See chapters 5 and 6 for information on programming protocols for probe control.)

Note: Because the thermal characteristics of a probe can never precisely match those of a sample, calculated control is often a better choice than probe control.

Customizing the Probe Vessel

For the most precise control of sample temperatures, install the probe's thermistor in the same type of tube that the samples will be placed in. This is particularly important when the sample tubes have much thicker walls than the probe's tube.

Follow these steps to customize the probe vessel:

1. Cut the hinge to the probe tube's lid, if there is one. Remove the lid and the attached the amber-colored thermistor.
2. Remove the lid from the new probe tube. Add oil to the probe tube as described below under "Adding the Oil."
3. Gently place the thermistor in the new tube, and snap the lid closed. Make sure that the lid from the original probe tube fits the new tube tightly . The probe wire may touch the sides of the tube. The thermistor should rest on the bottom of the tube.

Caution: The thermistor is extremely fragile. Handle it with great care.

Adding the Oil

Viscous oils are the best choice for the probe tube's representative sample. They closely mimic the thermal characteristics of buffer solution, which changes temperature sluggishly due to the high specific

heat of water. MJ RESEARCH recommends using heavy mineral oil, for the following reasons:

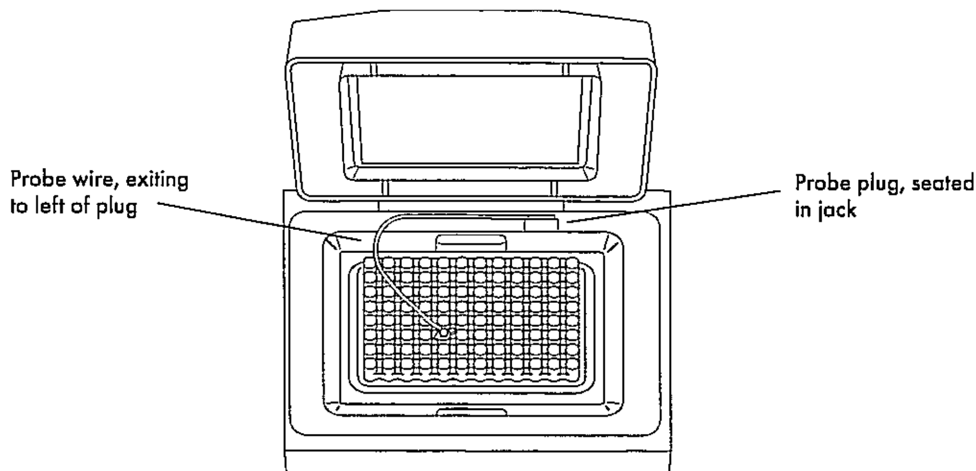
- The calculations required to determine the correct volume of oil are easy.
- It is widely available and inexpensive.

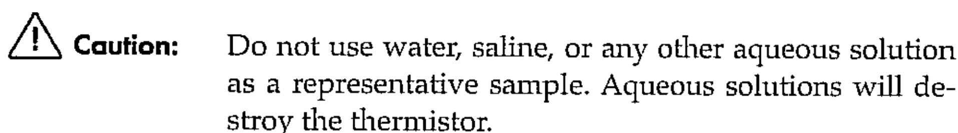
Add mineral oil to the probe tube in the following proportions: 1 x the volume of the buffer in an individual tube, plus 1 x the volume of oil overlay if one is used. **It is important to use the correct amount of oil, so that the representative sample changes temperature at the same rate as the actual samples.** To add the oil, open the sample tube and pipette in the appropriate amount. The oil must completely cover the thermistor.

Light and heavy silicone oil may also be used but necessitate more complex calculations to determine the amount to add to the probe tube. See chapter appendix 4-C for information on using these oils.

Note: Use only mineral oil or silicone oil as the representative sample. Do not use paraffin wax or Chill-out liquid wax, or the probe readings will not be accurate.

Figure 4-4 Correctly inserted probe.





Caution: Do not use water, saline, or any other aqueous solution as a representative sample. Aqueous solutions will destroy the thermistor.

Loading and Connecting the Probe

Seat the probe tube in the center of the block (fig. 4-4, p. 4-12). If oil is used to thermally couple samples to the block, it must also be used on the probe tube (but see "Using Oil to Thermally Couple Sample Vessels to the Block," p. 4-9).

Plug the probe into the jack at the back of the block, so that the wire is to the left of the plug (fig. 4-4).

Detecting a Faulty Probe

If the DNA Engine detects that the probe is broken or missing when a protocol begins running, the protocol's temperature control method is automatically switched from probe control to calculated control, and the following message is immediately displayed:

```
Run: CUSTOM1

Probe Sensor Failure
  Used Calc Control
```

If the probe malfunctions **during** a protocol run, the temperature control method is also switched to calculated control, and the run-time screen changes to say "Calc" instead of "Probe." When the run finishes, the following message is displayed:

```
Run: CUSTOM1

CALC control:
Probe not present
```

Appendix 4-A Tube, Microplate, and Sealing System Selection Chart

Key

● Reaction vessel fits block without modification.
○ Reaction vessel must be cut to fit this block.

MJ Research Thermal Cycler Blocks					Reaction Vessels		Sealing Options for Oil-Free Cycling						
96V (0.2ml)	48 (0.2ml)	60 (0.5ml)	30 (0.5ml)	16/16 slide	Description	MJ Research Catalog #	Microseal 'A' film MSA-5001	Microseal 'M' mat*	Microseal 'P' pad*	8-Strip caps TCS-0801	12-Strip caps TCS-1201	Self-Seal reagent SLR-0101	Frame-Seal chambers SLF-series
●	○				Multiplate 96-well microplates	MLP-9601	●	●	●	●	●		
●	●				Multiplate 48-well microplates	MLP-4801	●	●*	●*	●	○		
●	●				Multiplate 24-well microplates	MLP-2401	●	●*	●*	●	○		
●	●				Multiplate 25-well microplates	MLP-2501	●	●*	●*	○	○		
●					"Concord" 96-well microplates	CON-9601	●						
●	●				8-strip 0.2-ml tubes	TBS-0201	●	●*	●*	●	○		
●	○				12-strip 0.2-ml tubes	TBS-1201	●	●*	●*	○	●		
●	●				0.2-ml tubes, no caps	TBI-0201	●	●*	●*	●	●		
●	●				0.2-ml tubes w/caps	TWI-0201							
		●	●		0.5-ml tubes w/caps, thin wall	TBI-0501							
		●	●		0.5-ml tubes w/caps, thick wall	T6-1000							
				●	Glass slides	SLS-series						●	●

* Microseal 'M' and 'P' sealers are sized for 96-well blocks but can be used with fewer than 96 wells if the vessels are placed symmetrically in the block. Microseal 'P' pads are intended for use with Power Bonnet motorized lids.

Note: All tubes and multiplate microplates are made from polypropylene plastic, the optimal material for this application. "Concord" microplates are made from polycarbonate plastic, which is more prone to poor sealing and vapor leakage.

Appendix 4-B Safety Warning Regarding Use Of ^{35}S Nucleotides

Some researchers have experienced a problem with **radioactive contamination** when using ^{35}S in thermal cyclers. This problem has occurred with all types of reaction vessels.

The Problem

When ^{35}S nucleotides are thermally cycled, a volatile chemical breakdown product forms, probably SO_2. This product can escape the vessel and contaminate the sample block of a thermal cycler, and possibly the air in the laboratory. Contamination has been reported with microassay plates, 0.2-ml tubes, and 0.5-ml tubes.

96-Well Polycarbonate Microplates

These microplates present the largest risk of contamination. Polycarbonate is somewhat permeable both to water and the ^{35}S breakdown product. This problem is exacerbated when polycarbonate plates are held at high temperatures for long periods of time, or when the plates are sealed for oil-free thermal cycling.

0.2-ml Polypropylene Tubes and 96-Well Polypropylene Microplates

These tubes are manufactured with very thin walls to enhance thermal transfer. The thin walls are somewhat fragile and can "craze" or develop small cracks when subject to mechanical stress. Undamaged thin polypropylene tubes may also be somewhat permeable to the ^{35}S breakdown product. Either way, there have been reports of ^{35}S passing through the walls of 0.2-ml tubes of several different brands during thermal cycling. No data are yet available on radioactive contamination with polypropylene microplates.

0.5-ml Polypropylene Tubes

Contamination problems are rarer with this type of tube, but instances have been reported.

The Solution

1. Substitute the low-energy beta emitter ^{33}P in cycle sequencing. ^{33}P nucleotides are not subject to the same kind of chemical breakdown as ^{35}S nucleotides, and they have not been associated with volatile breakdown products.
2. If ^{35}S must be used, three things will help control contamination: an oil overlay inside the tubes, mineral oil in the thermal cycler outside the tubes, and use of thick-walled 0.5-ml tubes. Always run ^{35}S thermal cycling reactions in a fume hood, and be aware that vessels may be contaminated on the outside after thermal cycling. Please be certain that you are using the

appropriate detection methods and cleaning procedures for this isotope. Consult your radiation safety officer for his or her recommendations.

If mild cleaning agents do not remove radioactivity, harsher cleaners may be used. Users have suggested the detergent PCC-54 (Pierce Chemical Co., Rockford, Illinois; Pierce Eurochemie B.V., Holland), Micro Cleaning Solution (Cole-Parmer, Niles, Illinois), and Dow Bathroom Cleaner (available in supermarkets).

Caution: Harsh cleaning agents are corrosive to aluminum and must never be used on bare aluminum blocks. MJ RESEARCH blocks are anodized, so they have a protective coating of aluminum oxide. Still, harsh agents (such as those above) must be *thoroughly* rinsed away within a few minutes of application, or the anodization will degrade.

Appendix 4-C Using Silicone Oil in the Probe Tube

The following light and heavy silicone oils may be used instead of mineral oil as the representative sample in a probe tube:

- **Dow Corning #200 light silicone oil** (dimethypolysiloxane, Sigma #DMPS-5X)

  Density: 0.97g/ml

  Viscosity: 50cs

  Volume to use: 1.7 x volume of buffer in individual sample tube, plus one volume of oil overlay.

- **Dow Corning #200 heavy silicone oil** (dimethypolysiloxane, Sigma #DMPS-V)

  Density: 0.97g/ml

  Viscosity: 5cs

  Volume to use: 2.7 x volume of buffer in individual sample tube, plus one volume of oil overlay.

Note: Use these oils only in the proportions outlined above. Using them in any other proportion (for example, 1:1 with sample tube volumes) will lead to inaccurate sample heating.

5 Running Protocols

Running a Protocol, *5-2*
Choosing a Stored Protocol to Run, *5-2*
Choosing a Block to Run the Protocol On, *5-3*
Setting Up the Temperature Control Method, *5-3*
Setting Up a Block-Control Protocol, *5-3*
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Reading the Runtime Screen, *5-5*
Switching Between the Runtime Screen and the Main Menu, *5-6*
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Printing a Log for a Running Program, *5-7*
Manually Stepping Through a Protocol, *5-8*
Pausing a Running Protocol, *5-8*
Stopping a Running Protocol, *5-9*
Resuming a Protocol after a Power Outage, *5-9*
Using the Instant Incubation Feature, *5-10*

Running a Protocol

Running a protocol on the DNA Engine involves three steps:

1. Choosing a stored protocol to run
2. Choosing a block to run it on, if a dual-block Alpha unit is installed
3. Setting up the temperature control method

Either a custom-designed protocol or one of the factory-installed resident protocols may be run. See appendix E for descriptions of the resident protocols, which may be edited to fit your needs (see chapter 7). All the factory-installed protocols are stored in a single folder, called the <MAIN> folder, at the time of shipping.

Choosing a Stored Protocol to Run

With the Main Menu displayed, select *Run*, then press «Proceed». One of two types of screen will be displayed, depending on whether custom protocols have been stored in the <MAIN> folder or in custom folders:

- **If all protocols have been stored in the <MAIN> folder:**

  The first of three or more screens listing the protocols will be displayed. Custom protocols are listed first, then the 14 factory-installed programs:

```
Run:        <MAIN>
_CUSTOM1   CUSTOM2
 QUIKSTEP  2-STEP
 3-STEP    EXTEND
```

  Use the «Select» keys to scroll through the listed protocols. Scroll past the last- or first-listed protocol to see the next screen down or up. Select the desired protocol, then press «Proceed».

- **If custom protocols have been stored in one or more custom folders:**

  One or more screens listing all the folders residing in the machine will be displayed:

```
Run:
_<MAIN>    <FOLDER1>
 <FOLDER2> <FOLDER3>
```

  Select the folder that contains the protocol, then press «Proceed». One or more screens listing the protocols stored in the folder will be displayed. Use the «Select» keys to scroll through the listed protocols. Select the desired protocol, then press «Proceed».

In either instance, after you press «Proceed», a screen similar to the following example will be displayed:

```
Run: 2-STEP

Vessel Type:
   _TUBES     Plate
```

The top line of the screen will identify the selected protocol (2-STEP in the example). The other lines on the screen will request information needed to set up the temperature control method (explained below).

Choosing a Block to Run the Protocol On

If a dual-block Alpha unit is installed (or if networked Alpha units are available; see chapter 9), one of the blocks must be designated to run the protocol. The first available block is automatically designated when a protocol is chosen. Press «Block» to choose a different block. The selected block's letter is identified in the upper right-hand corner of the screen:

```
Run: 2-STEP    on  A

Vessel Type:
   _TUBES    Plate
```

The status indicator light for the selected block will flash green.

Setting Up the Temperature Control Method

When the protocol is selected (and the correct block is designated for a dual-block Alpha unit), one or more screens will be displayed. These screens will ask for information needed to set up the block's temperature control method. The DNA Engine can control the block's temperature in three different ways: **block control, calculated control,** and **probe control.** Chapter 6 explains in detail how these methods work and their implications for protocol design. The following describes how to respond to the screens that are displayed for each control method. When these screens have been dealt with, the protocol will begin to run.

Setting Up a Block-Control Protocol

A single screen asking about use of the heated lid will be displayed:

```
Run: ICEBUKET

Use heated lid?
      Yes  _NO
```

Select *Yes* or *No*, then press «Proceed». The protocol will begin running.

Setting Up a Calculated-Control Protocol

Three screens will be presented:

- A screen asking for sample vessel information:

```
Run: 2-STEP

Vessel Type:
   _TUBES   Plate
```

Select from the options shown (may include tubes, plates, and slides), then press «Proceed». Choose *Plate* for polycarbonate microplates and *Tube* for polypropylene tubes or polypropylene microplates. For blocks that hold only tubes, choose *Thin* or *Thick*, depending on the type of tube loaded.

Note: See "Using Calculated Control" in the *Slide Chambers Alpha Unit Operations Manual* for information on specifying slide format.

- A screen asking for the sample reaction volume (last line in example below):

```
Run: 2-STEP

Vessel Type: TUBES
Volume (µl):   10
```

Use the keypad to enter a sample volume in microliters, then press «Proceed». If sample vessels are sealed with oil or wax, include the volume of the oil or wax in the total sample volume entered.

Note: Specify 10µl for any volume less than 10µl.

Note: Verify that your vessel sealing system is adequately tight before performing 5-µl reactions.

- A screen asking about use of the heated lid. Select *Yes* or *No*, then press «Proceed». The protocol will begin running.

Setting Up a Probe-Control Protocol

A single screen asking about use of the heated lid will be displayed. Select *Yes* or *No*, then press «Proceed». If the probe is installed, the probe verification screen will be displayed:

```
Run: PROBE1

PROBE control:
Verify probe ready
```

Ensure that the probe is correctly installed (see "Loading and Connecting the Probe," chapter 4), then press «Proceed». The protocol will begin running.

If the probe is not installed, a warning message will be displayed:

```
RUN: PROBE1

CALC control:
  Probe not present
```

This screen says that since the probe is missing, the DNA Engine will run the protocol using calculated control if «Proceed» is pressed now. To avoid this, press «Cancel», install the probe, and set the protocol up again. When the probe verification screen is displayed, press «Proceed» to begin the protocol run.

Reading the Runtime Screen

During a protocol run a runtime screen will be displayed:

```
Run: 2-STEP
 1=  92.0˚ for 0:05
Cycle:  1
Calc:  68.0˚
```

The screen lists the protocol name (2-STEP in the example above), protocol step that is running (1), cycle number (1), method of temperature control (Calc), and the block temperature for block-control protocols, the calculated sample temperature for calculated-control protocols (68.0), and the probe temperature for probe-control protocols. When the step's target temperature is reached, a timer begins running in the lower right-

hand corner of the screen. The first digit is the minutes elapsed; the two digits after the colon are the seconds elapsed.

For protocols using calculated control or probe control, press the left «Select» key to display the block's temperature on the third line of the screen:

```
Run: 2-STEP
 1=  92.0˚ for 0:05
Block: 62.1
Calc:  68.0˚
```

This screen shows only as long as the left «Select» key is pressed. The runtime screen returns when you stop pressing the key.

Press the right «Select» key to see a screen listing the cycle number (if a GoTo step is executing), time elapsed so far for the protocol run, and estimated remaining time left in the run:

```
Run: 2-STEP
Cycle:   1
Total time:      0:20
Est remain: 1:01:51
```

This screen is also displayed only as long as the key is pressed. The runtime screen returns when you stop pressing the key.

Switching Between the Runtime Screen and the Main Menu

Press «Block» to toggle between the runtime display and the Main Menu. This allows you to edit a stored program, enter a new one, print a program, run another protocol on a dual-block Alpha unit or networked DNA Engine, or use the file utilities, while the protocol runs.

Reading the Protocol Completion Screen

When the protocol run finishes, a long beep sounds, and a notification screen is displayed:

```
Run: 2-STEP
  PROGRAM COMPLETE

Total time:    50:31
```

Certain error messages may also be displayed in this screen (see chapter 11). Press «Proceed» to return to the Main Menu.

Printing a Log for a Running Protocol

If the DNA Engine is connected to a printer, you can print a log for the run that includes

- The DNA Engine's serial number and software version
- The protocol's name
- The temperature control method for the protocol
- A list of the protocol's steps
- The runtime thermal data

To print a runtime protocol log, first ensure that a printer is connected to the machine. See "Printing a Program," chapter 8, for information about compatible printers.

Follow the instructions under "Choosing a Printer Port," chapter 8, to prepare the DNA Engine to communicate with your printer.

As long as a printer is connected to the machine, the following screen will appear whenever a protocol is run:

```
Run: 2-STEP

Printer output?
_YES   No
```

To print the protocol as it runs, select *Yes*, then press «Proceed».

Note: If no printer is connected to the machine, the screen allowing you to select printer output is not displayed at all.

Manually Stepping Through a Protocol

Once a protocol is running, pressing «Proceed» gives you the option of immediately advancing the protocol to the next programmed step, even if the machine is currently ramping the block's temperature (see chapter 6 for information on ramping). A confirmation screen will be displayed:

```
Run: 2-STEP

Go on to next step?
      _YES   No
```

Select *Yes*, then press «Proceed». The protocol will advance to its next step.

Pausing a Running Protocol

Press «Pause» to temporarily stop a running program. The timer in the lower right-hand corner of the runtime screen will be replaced by the word "Pause":

```
Run: 2-STEP
 1=  92˚ for 0:05
Cycle:  1
Calc:  92˚       PAUSE
```

The samples are held at the displayed temperature until either the «Pause» or the «Proceed» key is pressed, which causes the protocol run to resume.

A protocol cannot be paused before the target temperature for a given step has been reached. If «Pause» is pressed before this point, the block continues heating or cooling until the target is reached, and then the protocol is paused.

Stopping a Running Protocol

Press either «Stop» or «Cancel» to stop a running protocol. A cancellation confirmation screen will be displayed:

```
Run: 2-STEP

Stop 2-STEP?
       Yes  _NO
```

Select *Yes*, then press «Proceed» to cancel the protocol. The total run time for the protocol will be displayed:

```
Run: 2-STEP
   PROGRAM CANCELED

Total time:     1:15
```

Press «Proceed» to return to the Main Menu.

Note: Turning off the machine does not stop a running protocol. The DNA Engine will assume the protocol was stopped by a power outage and will resume running the protocol when the machine is turned back on (see below).

Resuming a Protocol after a Power Outage

If a power failure occurs when a protocol is running, the DNA Engine will hold the protocol in memory for a minimum of 24 hours to a maximum of 7 days, depending on environmental conditions.

When power is restored, the protocol will begin running again at the point at which it was stopped, and a notice about the power interruption will be displayed. The notice will identify the step and the cycle that were running when the power failure occurred, and the block's temperature at the time power was restored:

```
Run: 2-STEP
  A/C POWER FAILED
 Cycle 1    Step 1
Recovered at 20.2°
```

Press «Proceed» to remove this screen. The protocol's runtime screen will immediately be displayed.

Using the Instant Incubation Feature

The DNA Engine may be converted to a constant-temperature incubator by pressing «Instant Incubate». A screen allowing use of the heated lid will be displayed:

```
INCUBATE:

 Use heated lid?
     Yes  _No
```

Use the «Select» keys to enable or disable the heated lid, then press «Proceed». A screen allowing entry of the incubation temperature will be displayed.

```
INCUBATE:
 1=  20.0° for ever
 Use heated lid?
     Yes  _No
```

Use the keypad to enter any incubation temperature from –5.0°C to 105.0°C, then press «Proceed». The DNA Engine will incubate the sample at the specified temperature until «Cancel» or «Stop» is pressed.

When the sample block reaches the incubation temperature, a timer begins running in the lower right-hand corner of the screen. To stop and start the timer, press «Pause».

✔ **Tip:** The Pause feature is useful if you need to temporarily remove samples that must be incubated for a precise period of time. Pausing the timer while samples are not in the block allows you to track the exact duration of their incubation.

6 Creating Programs

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The Elements of a Program

DNA Engine programs consist of a series of steps encoding a protocol. These steps are run using one of three possible temperature control methods: calculated control, block control, and probe control.

Programs may contain five types of steps. Two of the steps are mandatory, and two are optional:

1. **Temperature step (mandatory):** Sets a temperature for the block and the length of time it is held at that temperature. The DNA Engine brings the block to this temperature at its maximum rate of heating or cooling, unless modifying instructions are added to the program. (The maximum rate of heating and cooling is up to 3°C/sec for all single- and dual-block Alpha units; 1.2°C/sec for the Slide Chambers Alpha unit.)
2. **Gradient step (optional):** allows you to program a temperature gradient across the sample block. The range of any single gradient can be as great as 24°C from left to right. The maximum programmable temperature is 105°C; the minimum programmable temperature is 30°C.
3. **Ramp step (optional):** Sets a slower-than-maximum rate of heating or cooling.
4. **GoTo step (optional):** Causes the program to cycle back to an earlier step for a specified number of times (up to 9,999 times).
5. **End step (mandatory):** Instructs the DNA Engine to shut down its heat pump because the program is complete.

Additional instructions, termed "options," can be added to certain program steps to modify their effects:

1. **Increment:** Modifies a temperature step to allow a progressive increase or decrease of temperature (0.1°–10.0°C per cycle) each time the step is executed in a cycle. This is useful in "touchdown" programs, when the annealing temperature of an oligonucleotide is not known.
2. **Extend:** Modifies a temperature step to allow progressive lengthening or shortening of a temperature step hold (by 1–60 sec/cycle) each time a step is executed in a cycle. This is useful for accommodating an enzyme with diminishing activity.
3. **Beep:** Modifies a temperature step or ramp step to make the machine beep when the target temperature is reached.

Designing a New Program

Translating a Protocol into a Program

Until you are completely familiar with programming the DNA Engine, you may find it helpful to first translate the protocol into DNA Engine program steps and options on paper. Write down the protocol to be programmed, one step per line. Then write the type of program step that goes with the protocol steps, at the end of each line. If a protocol step involves an option as well as a program step, write both names down on the same line. Finally, write the End step at the bottom of the list; programs will not run without this step. Number the lines 1 through N, where N is the final, End line.

Using the GoTo Step to Write Short Programs

The GoTo step allows programs of many repetitious steps to be shortened to just a few lines. When the program encounters a GoTo step, it returns to a specified step, repeats that step, and repeats all steps that follow, back to the GoTo step. When the program has returned, or cycled, back to the step a specified number of times, the program moves on to the step that follows the GoTo step.

For example, consider a basic cycle sequencing protocol consisting of 30 repeats of a denaturation, and an annealing/extension step. Rather than listing all 60 steps, use a GoTo step to design a short, easy-to-enter program:

Raw program:

1. 92° for 30 sec
2. 60° for 3 min
3. 92° for 30 sec
4. 60° for 3 min
5. 92° for 30 sec
6. 60° for 3 min
7. 92° for 30 sec

[continues for total of 60 lines]

DNA Engine program:

1. 92° for 30 sec
2. 60° for 3 min
3. GoTo step 1, 29 times (i.e., cycle back to step 1 and repeat steps 1 and 2, 29 times)
5. End

Choosing a Temperature Control Method

The DNA Engine can control block temperature in three possible ways, each of which has different implications for the speed and accuracy of sample heating:

- **Calculated control:** The DNA Engine adjusts the block's temperature to maintain samples of a specific volume in a specific vessel type

at programmed temperatures. This includes optimized "overshoots" of the block by a few degrees for a few seconds, which bring the samples to the programmed temperatures.

- **Block control:** The DNA Engine adjusts the block's temperature to maintain the block at programmed temperatures, independent of sample temperature.
- **Probe control:** The DNA Engine adjusts the block's temperature to maintain the probe at programmed temperatures.

Calculated Control

Calculated control is the method of choice for most types of programs, yielding the most consistent, most reliable, and fastest programs. When using calculated control, the DNA Engine maintains a running estimate of sample temperatures based on the block's thermal profile, the rate of heat transfer through the sample tube or slide, and the sample volume or mass (this information about the samples is provided when a program is run; see "Setting Up the Temperature Control Method," chapter 5). Since this estimate is based on known quantities and the laws of thermodynamics, sample temperatures are controlled much more accurately than with block or probe control.

Hold times can be shortened significantly when protocols are run under calculated control. In addition to the simple convenience of spending less time running reactions, shorter protocols also help preserve enzyme activity and minimize false priming. Cycling denaturations run under calculated control are usually optimal at 5 seconds. Annealing/extension steps can also be shortened, but the periods for these will be reaction specific.

Calculated control provides for shorter protocols in three ways:

1. Brief and precise block temperature overshoots are used to bring samples to temperature rapidly.
2. Incubation periods are timed according to how long the samples, not the block, reside at the target temperature.
3. The machine automatically compensates for vessel type and reaction volume.

Block Control

Block control provides less accurate control of sample temperatures than calculated control provides. Under block control, the temperature of samples always lags behind the temperature of the block. The length of the time lag depends on the vessel type and sample volume but typically is between 10 and 30 seconds. Block control is chiefly used to run protocols developed for other thermal cyclers that use block control.

Probe Control

Probe control is available for unusual circumstances that may require it. Ordinarily, though, it should be used with caution. While the DNA Engine will have no trouble heating the probe to the target temperature, if the probe is seated or prepared differently from the sample tubes, actual sample temperatures can vary widely from the probe's temperature. Probe control also cannot be used with microplates or slides.

Modifying Block- and Probe -Control Programs for Calculated Control

Probe-control programs will generally run well under calculated control, with no modification other than changing the method of temperature control. Block-control programs can be changed to calculated control by subtracting at least 15–20 seconds from each temperature step. Some empirical testing may be required to adjust modified programs for optimum performance.

Modifying a Program Designed for a Different Machine

The ramp programming step can be used to adapt programs designed for thermal cyclers with slower maximum heating and cooling rates than the DNA Engine. In addition, a given protocol will occasionally work better with a slower rate of temperature change; the ramp step can be used to optimize the program for such a protocol.

Entering a New Program

Programming the DNA Engine moves through five steps:

1. Initiating the program
2. Naming the program
3. Choosing a temperature control method
4. Entering the program's steps
5. Entering the End step

Each step involves entering values from the keyboard or making selections from a menu. Programs may be edited as they are being entered.

Programs are automatically saved when the End step is entered. They are stored in the <MAIN> folder unless folders have been created for them.

Initiating the Program

To initiate a new program, select *Enter* from the Main Menu, then press «Proceed». A naming screen will be displayed:

```
Enter:


Name: A
```

Naming the Program

Name the program an eight-character word consisting of any combination of letters (Roman and Greek), numbers, punctuation marks, or Japanese *Katakana*.

Press the right «Select» key to scroll forward and the left «Select» key to scroll backward through the alphabets and characters available, which are presented in this order: Roman alphabet, selected Greek letters, punctuation marks, numbers. To access the Japanese *Katakana* syllabary, press the «.» key. A second press of «.» returns the machine to Western characters.

When the character needed is displayed next to *Name*, press «Proceed». The character will be accepted, and the cursor will move one space to the right. Numbers and dashes may also be inserted by pressing the corresponding keys on the keypad.

When the name is complete, press «Proceed» once to accept the last character and again to accept the whole name. If the name is already in use for a program, a screen saying "Name In Use" will be displayed. If this happens, press «Proceed», then enter a different name.

Choosing a Temperature Control Method

When the name has been entered ("CUSTOM1" is used in the following and all succeeding examples), a screen requesting selection of a temperature control method will be displayed:

```
Enter: CUSTOM1
Control method:
   Block    Probe
  _CALCULATED
```

Select a control method, then press «Proceed».

Entering the Program's Steps

When a temperature control method has been chosen, the Enter Menu will be displayed:

```
Enter: CUSTOM1
Step 1= _TEMP
        Gradient
        Ramp  End
```

Use this menu to enter each step of the program:

- *Temp* enters a temperature step.
- *Gradient* enters a Gradient step.
- *GoTo* enters a GoTo step (note: since a protocol can not begin with a GoTo step,*GoTo* does not appear on the initial Enter Menu).
- *Ramp* enters a ramp step.
- *End* enters the End step.

Entering a Temperature Step

To enter a temperature step, select *Temp* from the Enter Menu, then press «Proceed». The first Temp screen will be displayed:

```
Enter: CUSTOM1
 1=

Temp °C:    _
```

The second line of this screen shows the number of the step being programmed (1 is used in the example above). The last line of the screen allows a target temperature (in degrees Celsius) to be entered for the step.

Use the keyboard to enter any number between –5.0 and 105.0 as the target temperature (92.0 is used in the example below):

```
Enter: CUSTOM1
 1=

Temp °C:  92.0
```

Press «Proceed». The target temperature will move to the second line of the screen, and a line allowing a hold time to be entered for the step will be displayed:

```
Enter: CUSTOM1
 1= 92.0°

Time:         _
```

Enter the hold time for the step (30 seconds is used in the example be-

low):

```
Enter: CUSTOM1
 1= 92.0˚

Time:        30
```

Note: If a hold time of zero (0) is entered, the DNA Engine will hold the block at the target temperature indefinitely.

Press «Proceed». The hold time will move to the second line of the screen, and a confirmation menu will be displayed on the last line:

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30

OK? _YES  No  Option
```

Select one of the displayed choices, then press «Proceed»:

- *Yes* accepts the step and displays the Enter Menu again. Use the Enter Menu to enter the next step in the program.
- *No* allows reentry of the target temperature and hold time for the step.
- *Option* displays the Options Menu (see "Modifying a Program Step with the Options," p. 6-11).

✔ **Tip:** Avoid programming many short holds of only a few seconds each. This can overheat the block, causing the "HS Overheating" or "PS Overheating" error messages to be displayed and triggering automatic Alpha unit shutdowns if the block exceeds its maximum allowable temperature.

Entering a Gradient Step

When you reach the step at which a gradient is desired, select *Gradient* from the Enter Menu and then press the «Proceed» key. For the purpose of this example the step in which the gradient will be entered shall be Step 2:

```
Enter: CUSTOM1
Step 2= Temp  Goto
        _GRADIENT
        Ramp  End
```

The gradient screen will appear:

```
Enter: CUSTOM1


Lower Temp °C:
```

Enter the lower limit temperature (for the purposes of this example, 50°), then press «Proceed». The upper temperature screen will appear:

```
Enter: CUSTOM1
        50.0°

Upper Temp °C:
```

Enter the upper temperature (for the purposes of this example, 70°), then press «Proceed». (Use integers only; decimals are not accepted).

The next screen requires you to enter a hold time for the temperature gradient step:

```
Enter: CUSTOM1
2= 50.0° to 70.0°

Time:
```

Enter the hold time in the form of min:sec. Press «Proceed». A confirmation screen will appear:

```
Enter: CUSTOM1
2= 50.0° to 70.0°
for 0:30
OK? Yes No Option
```

Select "Yes" to enter the step into memory and proceed to the next step; select "No" to reject or edit the current step. To preview, select *Option*, then *Preview*. *Preview* gives the predicted temperatures for each of the wells along the sample block's long axis. (The lines do not all appear in the display; use the «Select» keys to scroll up or down.)

```
Gradient Preview:

Column 1- 50.0° left
Column 2- 51.8°
Column 3- 53.6°
```

Editing a gradient step

To edit a gradient step, select *Edit* from the Main Menu. The program will be displayed as follows:

```
List: CUSTOM1
1= 92.0° for 0:20
2= 50.0° to 70.0°
       for 0:30
```

Use the «Select» keys to scroll to the step you want to edit.

Reviewing a gradient program

After the protocol has been run, the *Program Complete* screen will be displayed. Press the «Proceed» key, and the next screen will display a review of the gradient well temperatures:

```
Gradient Review:
Column 1-  50.0° left
Column 2-  50.5°
Column 3-  51.5°
Column 4-  53.2°
Column 5-  55.5°
Column 6-  58.4°
Column 7-  61.8°
Column 8-  64.6°
Column 9-  66.8°
Column 10- 68.4°
Column 11- 69.6°
Column 12- 70.0° right
```

Scroll up and down the screen using the «Select» keys. When scrolling the "Gradient Review" line will be held constant while the lower 3 lines can be manipulated up or down. The temperatures displayed will be for each of the twelve wells along the long-axis of the sample block. These temperatures will be predicted from an algorithm as a steady-state temperature of the block and the sample. You may then return to the main menu by pressing the «Proceed» key.

Using the gradient calculator

The gradient calculator predicts, for a given gradient, the temperatures for each of the twelve wells along the long-axis of the sample block. These temperatures will be predicted from an algorithm as a steady-state temperature of the block and the sample. To use the gradient calculator, select the *List* command from the main menu and then press the «Proceed» key. You will see a *List* screen:

```
List:
  Programs
  _GRADIENT CALCULATOR
```

Select *Gradient Calculator* and press the «Proceed» key :

```
Lower Temp °C:  50.0°
Upper Temp °C:  70.0°
```

Enter lower limit temperature of the gradient using the number keys and then press the «Proceed» key. Enter the upper temperature for the gradient using the number keys and «Proceed» key. You may enter integers without decimal points. The following screen will be displayed:

```
Gradient Calculator:
Column 1-  50.0° left
Column 2-  50.5°
Column 3-  51.5°
Column 4-  53.2°
Column 5-  55.5°
Column 6-  58.4°
Column 7-  61.8°
Column 8-  64.6°
Column 9-  66.8°
Column 10- 68.4°
Column 11- 69.6°
Column 12- 70.0°right
```

You may scroll up and down the screen using the «Select» keys. When scrolling, the "Gradient Calculator" line will be held constant while the lower 3 lines can be manipulated up or down.

Entering a Ramp Step

To enter a ramp step, select *Ramp* from the Enter Menu. The first Ramp screen will be displayed:

```
Enter: CUSTOM1
 3=

Rate ˚C / s: _
```

The second line of this screen shows the number of the step being programmed (3 is used in the example above). The last line of the screen allows a ramp rate (in degrees Celsius per second) to be entered for the step.

Use the keyboard to enter any rate up to 2.5°C/sec (1°C/sec is used in the example below):

```
Enter: CUSTOM1
 3=

Rate ˚C / s: 1
```

Note: If a ramp rate faster than the Alpha unit's maximum rate of heating and cooling is entered, the maximum rate will be used.

Press «Proceed». The ramp rate will move to the second line of the screen, and a line allowing entry of a finish temperature for the ramp step will be displayed:

```
Enter: CUSTOM1
 3= 2.0˚/s

Finish temp:    _
```

Enter the finish temperature for the ramp step (69°C is used in the example below):

```
Enter: CUSTOM1
 3= 2.0˚/s

Finish temp:  69
```

Press «Proceed». The finish temperature will be moved to the second line of the screen, and a confirmation menu will be displayed on the last line:

```
Enter: CUSTOM1
 3= 2.0˚/s to  69.0˚

OK? _YES  No  Option
```

Select one of the displayed choices, then press «Proceed»:

- *Yes* accepts the step and displays the Enter Menu again. Use the Enter Menu to enter the next step in the program.
- *No* allows reentry of the ramp rate and finish temperature.
- *Option* displays the Options Menu (see "Modifying a Program Step with the Options," p. 6-11).

✔ **Tip:** A ramp step will not hold the block at its finish temperature. Instead, as soon as finish temperature is reached, the next step of the program will immediately be executed. To hold the block for some period of time at a ramp step's finish temperature, make sure a temperature step immediately follows the ramp step, and program the hold at the finish temperature into this step.

Entering a GoTo Step

To enter a GoTo step, select *GoTo* from the Enter Menu. The first GoTo screen will be displayed:

```
Enter: CUSTOM1
 5=GoTo

GoTo step: _
```

The second line of this screen shows the number of the step being programmed (5 is used in the example above). The last line of the screen allows entry of the number of the step the program should cycle back to.

Enter the number of the step the program should cycle back to (1 is used in the example below):

```
Enter: CUSTOM1
 5=GoTo

GoTo step: 1
```

Press «Proceed». The step number will move to the second line of the screen, and a line allowing an additional number of cycles to be entered will be displayed:

```
Enter: CUSTOM1
 3=GoTo 1

Addtnl cycles:    _
```

Enter the additional number of times the program should cycle back to the step (3 is used in the example below):

```
Enter: CUSTOM1
 3=GoTo 1

Addtnl cycles:    3
```

Press «Proceed». The number of additional cycles will move to the second line of the screen, and a confirmation menu will be displayed on the last line:

```
Enter: CUSTOM1
 3= GoTo 1,  3 times

OK? _YES  No
```

Select one of the displayed choices, then press «Proceed»:

- *Yes* accepts the step and displays the Enter Menu again. Use the Enter Menu to enter the next step in the program.
- *No* allows reentry of the step number and number of additional cycles.

Entering the End Step

To enter the End step, select *End* from the Enter Menu. The single End screen will be displayed:

```
Enter: CUSTOM1
 4=End

OK? _YES  No
```

This screen automatically enters "End" on on the second line of the screen, next to a step number, and displays a confirmation menu for the step on the last line of the screen.

Select one of the displayed choices, then press «Proceed»:

- *Yes* accepts the step, stores the program, and displays the Main Menu.
- *No* displays the Enter Menu so that additional steps can be added.

If you have created custom folders for your programs (see chapter 8), choosing *Yes* brings up a screen listing the folders:

```
Save program in:
<MAIN>  <FOLDER1>
<FOLDER2>
```

Select the folder you want to store the program in, then press «Proceed». The program will be stored in the folder, and the Main Menu will be displayed.

Modifying a Program Step with the Options

The Options Menu is accessible from the confirmation menus of temperature steps and ramp steps. To access the Options Menu, select *Option* from the confirmation menu of a temperature or ramp step, then press «Proceed». The Options Menu will be displayed on the bottom line of the screen:

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30

Option:_INC Ext Beep
```

- *Inc* modifies a temperature step with an increment option. An increment option allows a progressive increase or decrease of temperature each time the step is executed in a GoTo cycle.
- *Ext* modifies a temperature step with an extend option. An extend option allows a progressive lengthening or shortening of hold times each time the step is executed in a GoTo cycle.
- *Beep* modifies a temperature step or a ramp step, causing the machine to beep when a specified target temperature is reached.

Entering an Increment Option

To enter an increment option, select *Inc* from the Options Menu for a temperature step, then press «Proceed». The first Inc screen will be dis-

played:

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30
    +
˚C / cycle:  + _
```

The temperature step being modified appears on the second line of this screen. The plus sign on the third line means that the screen is set up to enter a progressive **increase** in temperature per cycle. Press «–» to switch to a minus sign, allowing entry of a progressive **decrease** in temperature. Press «Cancel» to change back to a plus sign.

Enter the numerical value of the temperature increase or decrease (1.2 is used in the example below):

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30
    +
˚C / cycle:  + 1.2
```

Press «Proceed». The Inc value just entered will move to the third line of the screen, and a confirmation menu will be displayed on the last line:

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30
    + 1.2˚ per cycle
OK? _YES  No  Option
```

Select one of the displayed choices, then press «Proceed»:

- *Yes* accepts the Inc value and displays the Enter Menu again. Use the Enter Menu to enter the next step in the program.
- *No* allows reentry of the Inc value.
- *Option* displays the Options Menu again. Use the Options Menu to enter another option for the step.

Entering an Extend Option

To enter an extend option, select *Ext* from the Options Menu of a temperature step, then press «Proceed». The first Ext screen will be displayed:

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30
    +
Sec / cycle:  +  _
```

As for Inc, the temperature step being modified appears on the second line of this screen. The plus sign on the third line means that the screen is set up to enter progressive lengthening of hold time. Press «–» to switch to a minus sign, allowing entry of a progressive shortening of hold time. Press «Cancel» to change back to a plus sign.

Enter the numerical value of the increase or decrease in hold time (1.0 is used in the example below):

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30
    +
Sec / cycle:  +  1
```

Press «Proceed». The Ext value just entered will move to the third line of the screen, and a confirmation menu will be displayed on the last line:

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30
    +  1 sec / cycle
OK? _YES  No  Option
```

Select one of the displayed choices, then press «Proceed»:

- *Yes* accepts the Ext value and displays the Enter Menu again. Use the Enter Menu to enter the next step in the program.
- *No* allows reentry of the Ext value.
- *Option* displays the Options Menu again. Use the Options Menu to enter another option for the step.

Entering a Beep Option

To enter a beep, select *Beep* from the Options Menu for a temperature step or a ramp step, then press «Proceed». The word "Beep" will be displayed on the third line of the screen, and a confirmation menu will be displayed on the last line:

```
Enter: CUSTOM1
 1=  92.0˚ for  0:30
    Beep
OK? _YES  No  Option
```

Select one of the displayed choices, then press «Proceed»:

- *Yes* accepts the Beep option and displays the Enter Menu again. Use

the Enter Menu to enter the next step in the program.

- *No* cancels the Beep option.
- *Option* displays the Options Menu again, if a temperature step is being modified. Use the Options Menu to enter another option for the step. (*Option* will not be displayed if a ramp step is being modified, since Beep is the only option available for ramp steps.)

Revising During Programming

To change values in a program you are entering, follow the procedures described below. This editing method should be used to change just a few values at a time. To make many changes, or to delete or add entire steps, use Edit mode (see chapter 7).

To Change the Last Value Entered or Menu Option Chosen

Press «Cancel». The choice just made will be cancelled, so that another value may be entered or another menu option chosen. Press «Proceed» after changing a value, so that the program will accept it.

To Change All the Values in the Step Being Entered

Repeatedly press «Cancel». Each time you press the key, the cursor will move backward through the values for the step, deleting the values as it moves. When all values for the step have been deleted, enter new values, or press «Cancel» one more time to display the Enter Menu for the step. At this point you can change the step to a different type.

To Change Values for Earlier Steps in the Program

Repeatedly press «Cancel» until the Enter Menu for the step you are working on is displayed. Press «Cancel» one more time to display a list of all steps entered so far. The list will show the step number and the values for each step, including any options chosen. The last-entered steps will be displayed first:

```
Enter: CUSTOM1
 3=  55.0˚ for  0:30
 4=  72.0˚ for  1:00
 5=
```

At this point, you can choose to change selected values in program steps, to add an option, to enter Edit mode, or to delete the entire program.

Press the «Select» keys to scroll through the program. The left «Select» key moves the cursor backward through the steps, and the right «Select» key moves it forward.

- **To change selected values or add an option:** To change a value, move the cursor to it, then press «Cancel». Enter the new value, then press «Proceed». If you change your mind, press «Cancel» again; the deleted value will be restored.

  To add an option to a step, move the cursor to the step number, then press «Proceed». The Edit Menu will be displayed:

```
Enter: CUSTOM1.
 2=   92.0° for 0:40
      _EDIT   Insert
Step: Option  Delete
```

  Select *Option*, then press «Proceed», and follow the procedure for adding an option. To abandon the Edit Menu and return to the list of program steps, press «Cancel».

- **To enter Edit mode:** Move the cursor to the number of the step you wish to edit, then press «Proceed». See chapter 7 for information on entering Edit mode. If you do not wish to enter Edit mode, press «Cancel». The screen will return to the list of program steps.

- **To delete the program:** Position the cursor on any step number and press «Cancel». A cancellation confirmation screen will be displayed.

```
Enter: CUSTOM1

Cancel program?
     _YES   NO
```

  Select *Yes*, then press «Proceed». The program will be deleted, and the Main Menu will be displayed.

Deleting a Program

To delete a program after at least one complete step has been entered, see "Revising During Programming," (p. 6-14).

To delete a program before one complete step has been entered, repeatedly press «Cancel». The cursor will move backward through the step's

values, deleting them as it goes. When all values have been deleted, keep pressing «Cancel» to move backward through Enter mode screens until the Main Menu reappears, at which point you can begin a new program.

Keeping a Permanent Record of Programs

Occasionally in the course of repairing a defective DNA Engine, it is necessary to replace the chip that stores all custom user protocols. To avoid losing your protocols in such an event, always maintain an up-to-date record of them. Protocols may be printed out using the List utility (see chapter 8), stored on a computer using the DNA Engine Driver Software, or written down in a notebook.

7 Editing Programs

Editing a Stored Program, *7-2*
    Initiating Editing, *7-2*
    Editing the Program, *7-3*
        Inserting a New Step, *7-4*
        Deleting a Step, *7-4*
        Adding an Option, *7-4*
Saving an Edited Program, *7-4*
Cancelling Editing Changes, *7-5*

Editing a Stored Program

The DNA Engine's editing tools, available through *Edit* on the Main Menu, make it easy to extensively edit stored programs by

- Changing individual values in program steps,
- Adding new steps,
- Deleting steps, and
- Adding options to temperature and ramp steps.

Note: The editing tools do not include a renaming function. To rename a program, see "Renaming a Program," chapter 8.

Initiating Editing

To initiate editing, select *Edit* from the Main Menu, then press «Proceed». One of two types of screen will be displayed, depending on whether your programs have been stored in the <MAIN> folder or in custom folders.

- **If all programs have been stored in <MAIN>:**

  The screen will list the contents of <MAIN>:

```
Edit:       <MAIN>
_CUSTOM1    CUSTOM2
 CUSTOM3    QUIKSTEP
 2-STEP     3-STEP
```

  Select the program to be edited, then press «Proceed».

- **If programs have been stored in custom folders:**

  The screen will list all the folders residing in the machine:

```
Edit:
_<MAIN>   <FOLDER1>
 <FOLDER2>
```

  Select the folder containing the program, then press «Proceed». A list of all programs in the folder will be displayed. Select the program to be edited (CUSTOM1 is used in the following and all succeeding examples in this chapter), then press «Proceed».

In either instance, after you press «Proceed», the first editing screen will be displayed (see next section).

✔ **Tip:** To retain the original version of a program, copy the program (see "Copying a Program," chapter 8), and then edit the copy.

Editing the Program

The first editing screen displays all three temperature control methods. The program's current temperature control method is displayed in all-capital letters:

```
Edit: CUSTOM1
Control method:
    Block    Probe
    _CALCULATED
```

Select a different temperature control method if desired, then press «Proceed» The first three lines of the program will be displayed:

```
Edit: CUSTOM1
_1=  92.0˚ for  0:10
 2=  63.0˚ for  1:00
 3=GoTo 1,  24 times
```

Use the «Select» keys to scroll up and down through the program. The cursor will progressively move to the step number and the individual values for each step.

To change an individual value in a step, position the cursor on it and type the new value, then press «Proceed». The new value will be displayed on the screen. To cancel a change, press «Cancel». The original value will be restored.

To add or delete a step, or to modify a step with an option, position the cursor on the step number, then press «Proceed». The Edit Menu will be displayed for that step:

```
Edit: CUSTOM1
 2=  63.0˚ for  1:00
      _EDIT   Insert
Step: Option  Delete
```

- *Edit* displays the previous screen again, but with the cursor positioned on the first value for the step, so that it may be changed.
- *Insert* allows a step to be added **before** the displayed step.
- *Delete* deletes the displayed step.
- *Option* allows an option to be added to the displayed step if it is a temperature or a ramp step.

Inserting a New Step

To insert a new step, select *Insert* from the Edit Menu, then press «Proceed». The Enter Menu will be displayed for the new step (a new step 2 is added in the example below):

```
Edit: CUSTOM1

Step 2= _TEMP  GoTo
        Ramp   End
```

Use the Enter Menu to create the new step (see "Entering the Program's Steps," chapter 6). When the step is complete, select *Yes* from the confirmation menu, then press «Proceed». The program being edited will be displayed again, with the new step appearing among the listed steps.

Deleting a Step

To delete a step, select *Delete* from the Edit Menu, then press «Proceed». The step will immediately be deleted, and the program being edited will be displayed again, minus the deleted step.

To cancel a deletion, see "Cancelling Editing Changes," p. 7-5.

Note: Be careful when using *Delete*. Once a step has been deleted, it cannot be recovered without abandoning all editing changes that have been made in the program. This could be inconvenient if the program has been extensively edited.

Adding an Option

To add an option to a step, select *Option* from the Edit Menu, then press «Proceed». The option menu will be displayed for the step. Add the desired option to the step (see "Modifying a Program Step with the Options," chapter 6). When the option is complete, select *Yes* from the confirmation menu, then press «Proceed». The steps of the program being edited will be displayed again, with the new option appearing in the list.

Saving an Edited Program

To save an edited program, use the right «Select» key to scroll to the End step of the program. Position the cursor on the number for the step, then

press «Proceed». A line allowing the editing session to be ended will be displayed on the last line of the screen:

```
Edit: CUSTOM1
 2=End

Step:  _END   Insert
```

- *End* saves the changes and displays the Main Menu. This ends the editing session.
- *Insert* allows another step to be added just before the End step.

Cancelling Editing Changes

To cancel all editing changes made to a program, use the «Select» keys to move the cursor to any step number, then press «Cancel». A cancellation confirmation screen will be displayed:

```
Edit: CUSTOM1

Cancel changes?
      _YES   No
```

Select *Yes*, then press «Proceed». All editing changes will be abandoned, and the Main Menu will be displayed.

8 Using the Utilities

Locating a Stored Program, *8-2*
File Utilities, *8-2*
Creating a Folder, *8-3*
Assigning a Password to a Folder, *8-3*
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Renaming a Program, *8-5*
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List Utilities, *8-6*
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Choosing a Remote Port, *8-7*
Choosing a Printer Port, *8-8*
Choosing a Minimum Block Temperature for the Hot Bonnet, *8–9*
Choosing a Temperature Control Method for the Hot Bonnet, *8-9*
Determining the Software Version Number, *8-10*

Locating a Stored Program

Many of the DNA Engine's utilities require you to locate a program stored in the machine. The actions necessary to do this depend on whether your programs have been stored in the <MAIN> folder or in custom folders.

- **If all programs have been stored in <MAIN>:**

  The screen will list all the programs that <MAIN> contains:

  ```
  Copy:       <MAIN>
  _CUSTOM1    CUSTOM2
   QUIKSTEP   2-STEP
   3-STEP     EXTEND
  ```

  Select the desired program from this list.

- **If programs have been stored in custom folders:**

  The screen will list all the folders residing in the machine:

  ```
  Copy:
  <MAIN>     <FOLDER1>
  <FOLDER2>  <FOLDER3>
  ```

  Select the folder containing the program, then press «Proceed». A list of all programs in the folder will be displayed. Select the desired program, then press «Proceed».

File Utilities

Use these utilities, available from the Files Menu, to accomplish the following tasks:

- Create folders to store programs in
- Assign passwords to folders
- Copy, delete, rename, or move programs

To display the Files Menu, select *Files* from the Main Menu, then press «Proceed»:

```
Files:
_COPY      Delete
 Move      Rename
 Folder    Secure
```

Creating a Folder

The DNA Engine's memory can hold up to 11 folders, including the <MAIN> folder. New programs are placed in the <MAIN> folder by default unless a different folder is specified.

To create a folder, select *Folder* from the Files Menu, then press «Proceed». A naming screen will be displayed:

```
New Folder:

Name: A
```

Name the folder, using the instructions found under "Naming the Program" in chapter 6, then press «Proceed». The name will be assigned to the new folder, the folder will be stored, and the Main Menu will be displayed.

Assigning a Password to a Folder

Protocols in a password-protected folder cannot be edited, renamed, or deleted, nor can new protocols be placed in the folder without the password. Users without knowledge of the password can still run, copy, and view a program.

Note: A password cannot be assigned to the <MAIN> folder.

To assign a password to a folder, select *Secure* from the Files Menu, then press «Proceed». A list of all folders in the machine will be displayed. Select the desired folder (other than <MAIN>), then press «Proceed». The password assignment screen will be displayed:

```
Secure: <FOLDER1>

New password: _
```

Valid passwords consist of numbers up to four digits long. For passwords of three digits or less, press «Proceed» after the password has been entered. Passwords four digits long will be automatically accepted as soon as typed, and the Main Menu will be displayed.

Passwords can be changed at any time. Follow the steps described above to select the desired folder, then press «Proceed». A screen asking for the old password will be displayed:

```
Secure: <FOLDER1>


Old password: _
```

Enter the old password, then press «Proceed». The password assignment screen be displayed. Enter the new password, then press «Proceed». The new password will be assigned to the folder, and the Main Menu will be displayed again.

Deleting a Folder

A folder must be empty before it can be deleted. After all programs have been moved or deleted from the folder, select *Delete* from the Files Menu, then press «Proceed». A list of all folders in the machine will be displayed. Select the folder to be deleted, then press «Proceed». A confirmation screen will be displayed:

```
Delete: <FOLDER1>

Delete empty folder?
     -YES   No
```

Select *Yes*, then press «Proceed». The folder will be deleted, and the Main Menu will be displayed.

To cancel the deletion, press «Cancel», or select *No* and press «Proceed».

Copying a Program

The copy utility copies a program and gives the copy a new name. Copies can be placed in the original folder or a new one.

To copy a program, select *Copy* from the Files Menu, then press «Proceed». Locate the program to be copied (see "Locating a Stored Program," p. 8-2), then press «Proceed». If more than one folder of programs is present in the machine, a screen allowing you to specify the folder the program will be copied to will be displayed:

```
Copy CUSTOM1 to:
_<FOLDER1> <FOLDER2>
 <FOLDER2>
```

Select a folder to copy the program to, then press «Proceed». A naming screen will be displayed:

```
Copy CUSTOM1 to:


New name: A
```

Name the copied program (see "Naming the Program," chapter 6), then press «Proceed». The program will be copied to the specified folder under the new name, and the Main Menu will be displayed.

Renaming a Program

To rename a program, select *Rename* from the File Menu, then press «Proceed». Locate the program to be renamed (see "Locating a Stored Program," p. 8-2), then press «Proceed». A naming screen will be displayed:

```
Rename: CUSTOM1


New name: A
```

Name the new program (see "Naming the Program," chapter 6), then press «Proceed». The program will be renamed and stored, and the Main Menu will be displayed.

Moving a Program

To move a program, select *Move* from the Files Menu, then press «Proceed». Locate the program to be moved (see "Locating a Stored Program," p. 8-2), then press «Proceed». A screen listing all folders will be displayed. Select the folder the program should be moved to, then press «Proceed». The program will be moved to the new folder, and the Main Menu will be displayed.

Deleting a Program

To delete a program, select *Delete* from the Files Menu, then press «Proceed». Locate the program to be deleted (see "Locating a Stored Program," p. 8-2), then press «Proceed». A confirmation screen will be displayed:

```
Delete:  CUSTOM3

Delete program?
     _YES   No
```

Select *Yes*, then press «Proceed». The program will be deleted, and the Main Menu will be displayed.

To cancel the deletion, press «Cancel», or select *No* and press «Proceed».

List Utilities

Use these utilities, available under List, to accomplish the following task:

- View and print program steps

Viewing a Program in the LCD Window

To view a program in the LCD window, select *List* from the Main Menu, then press «Proceed». Locate the program to be viewed (see "Locating a Stored Program," p. 8-2), then press «Proceed». What happens next depends on whether a printer is connected to the DNA Engine.

- If a printer is connected:

  A screen will be displayed asking whether the program should be sent to the printer:

  ```
  List: CUSTOM1

  Printer output?
        Yes  _NO
  ```

  Select *No*, then press «Proceed». The program will be listed in the LCD window. The first listed screen will show the program's control method. Press «Proceed» to view subsequent screens listing each program step, including any options. Use the «Select» keys to scroll up or down through the steps one line at a time. To return to the Main Menu, press «Cancel» at any time, or press either the right «Select» key or «Proceed» when the last step appears on the screen.

- If a printer is not connected:

  The program will immediately be listed in the LCD window. You will not be given the option of printing at all.

Note: Listed programs cannot be edited. To edit programs, select *Edit* from the Main Menu (see chapter 7).

Printing a Program

First ensure that the DNA Engine is connected to a printer. If no printer is connected, you will not be given the option of printing at all. The DNA Engine's parallel interface is compatible with most serial and laser printers, the Epson® LX-810 dot-matrix printer parallel interface, and dot-matrix printers using interfaces similar to the Epson's. Use a standard DB-25/Centronics parallel interface cable to connect the DNA Engine's parallel port to the printer's parallel port. A null-modem serial cable will usually be required to connect the DNA Engine's serial port to a printer's serial port.

Use the Setup utilities to choose a printer port for the DNA Engine (see "Choosing a Printer Port," p. 8-8).

Select *List* from the Main Menu, then press «Proceed». Locate the program to be printed (see "Locating a Stored Program," p. 8-2), then press «Proceed». The screen asking whether the program should be sent to the printer will be displayed (see above). Select *Yes,* then press «Proceed». The program will be printed.

Setup Utilities

Use these utilities, available from the Setup Menu, to accomplish the following tasks:

- Choose a remote port and a printer port
- Choose a temperature control method for the Hot Bonnet's heated lid
- Look up the DNA Engine's software version number

To display the Setup Menu, select *Setup* from the Main Menu, then press «Proceed»:

```
Setup:
_REMOTE    Printer
 Lid       Version
```

Choosing a Remote Port

To choose either the serial port or the IEEE-488 port, select *Remote* from the Setup Menu, then press «Proceed». The port selection screen will be displayed:

```
Remote:
 Serial   _IEEE-488
```

The cursor will be positioned at the port currently in use.

To choose the serial port, select *Serial*, then press «Proceed». The cursor will move to *Serial*, and a line allowing a baud rate to be specified will be displayed on the last line of the screen:

```
Printer:
_SERIAL    IEEE-488

Baud: 9600 2400_1200
```

Select a baud rate, then press «Proceed». The remote port assignment will change to serial, and the Main Menu will be displayed.

To choose the IEEE-488 port, select *IEEE-488*, then press «Proceed». The cursor will move to *IEEE-488*, and a line allowing the DNA Engine to be assigned an IEEE-488 address will be displayed on the last line of the screen:

```
Printer:
 Serial   _IEEE-488

Address (0-30): 0
```

The machine's current address will be displayed. To change it, enter a number from 0–30, then press «Proceed». The remote port assignment will change to IEEE-488, the address number will be assigned to the machine, and the Main Menu will be displayed.

Note: Do not choose 0 for the machine address unless the DNA Engine will be a controller in a network (see chapter 9).

Choosing a Printer Port

To choose a printer port, select *Printer* from the Setup Menu, then press «Proceed». The port selection screen will be displayed:

```
Printer:
 Parallel _SERIAL
```

Select the desired port, then press «Proceed». If serial was chosen, specify the printer's baud rate as for "Choosing a Remote Port" (p. 8-7).

Choosing a Minimum Block Temperature for the Hot Bonnet

To set a minimum block temperature below which the Hot Bonnet will automatically turn off, select *Lid* from the Setup Menu, then press «Proceed». The following screen will be displayed:

```
Lid:

Mode      _MINIMUM
```

Select «MINIMUM» and press «Proceed». The following screen will be displayed:

```
Lid Minimum:

   Turn off heated
    lid below: 23°C
```

Use the keypad to enter the temperature, then press «Proceed» to enter the value and return to the main menu.

Choosing a Temperature Control Mode for the Hot Bonnet

Two temperature control modes are available for the Hot Bonnet:

- **Tracking:** Offsets the temperature of the heated inner lid at least a specified number of degrees Celsius in comparison to the temperature of the sample block.
- **Constant:** Keeps the inner lid at a specified temperature (°C).

The DNA Engine is set for the tracking mode at the factory, using an offset of 5°C, which should be adequate for most reactions. The constant mode is provided for unusual reactions requiring the inner lid to be hot at all times. When using the constant mode, specify a lid temperature at least 10°C higher than any temperature used in the protocol; we suggest 105°C.

To choose a control mode, select *Lid* from the Setup Menu, then press «Proceed». The following screen will appear.

```
Lid:

_MODE  Minimum
```

Select «Mode», then press «Proceed» (to select a minimum block temperature, see "Choosing a Minimum Block Temperature for the Hot Bonnet," above).

The mode currently in use will be selected in the screen; select a different control mode if desired, then press «Proceed» to enter the mode and return to the main menu.

If the tracking mode is chosen, a lid offset (up to 45°C above the block, but not to exceed 110°C) must be specified. A line for this will appear at the bottom of the screen: "Lid offset °C: 15." If the constant mode is chosen, a lid target temperature (up to 110°C) must be specified. A line for this will appear at the bottom of the screen: "Lid target °C: _." In either case, enter a number, then press «Proceed». The tracking control mode will be implemented, and the Main menu will be displayed.

Determining the Software Version Number

At times it is necessary to determine the version number of the software installed in the DNA Engine (e.g., to report a problem to MJ Research). To do this, select *Version* from the Setup Menu, then press «Proceed». The current version number will be displayed:

```
Version:          1.1L

 CTRL  L     EXEC  L
 USER  L     COMM  L
```

The top line reports the software version number (1.1L in the example). The other two lines refer to the four "pages" that the software has been broken into and their associated versions (L in the example).

MJ Research periodically updates the DNA Engine's software to incorporate new features or allow new Alpha units to be used. Most upgrades are available free of charge for units under warranty and may be installed into a DNA Engine electronically from a desktop computer. Contact your MJ Research sales representative or an authorized distributor for details. Occasionally upgrades may require a hardware change. These upgrades require return of the DNA Engine base to MJ Research or an authorized distributor.

9 Networking

Overview of Networking, *9-2*
Networking Machines Without a Computer, *9-2*
Connecting the Machines, *9-2*
Assigning Address Numbers to Machines, *9-2*
Running a Protocol on Networked Machines, *9-3*
Networking Machines with a Computer, *9-4*
Creating a Computer-Controlled IEEE-488 Network, *9-5*
Creating a Computer-Controlled RS-232 Network, *9-5*

Overview of Networking

Up to 15 machines consisting of any combination of DNA Engines and DNA Engine Tetrads may be networked, with or without a computer as the controller. When networking with a computer, all the machines are controlled from the computer's keyboard. When networking without a computer, a specially designated DNA Engine controls the other machines in the network. Any combination of single- and dual-block Alpha units may be loaded into networked machines, and different protocols can be run independently on them.

Networked machines can help save time for a busy laboratory. For example, in a DNA Engine–controlled network, protocols can be entered into the controlling machine and then be run on all the other machines connected to the network.

Networking Machines Without a Computer

Connecting the Machines

Connect DNA Engines and DNA Engine Tetrads via the IEEE-488 port on the back of each machine (see figs. 2-3 and 12-4), using high-quality cables. Although several connection configurations are possible, we recommend a simple daisy chain or star pattern. The DNA Engine's IEEE-488 interface is fully compatible with both the IEEE-488.2 and the IEEE-488.1 standards.

Assigning Address Numbers to Machines

Each machine must have a unique address number, which is assigned from its control panel. Address numbers cannot be assigned from the controller.

To assign an address number to a machine, select *Setup* from the Main Menu, then press «Proceed». The Setup Menu will be displayed:

```
Setup:
_REMOTE    Printer
 Lid       Version
```

Select *Remote*, then press «Proceed». A screen allowing choice of a port will be displayed:

```
Remote:
_SERIAL  IEEE-488
```

Select *IEEE-488*, then press «Proceed». The remote addressing screen will be displayed:

```
Remote:
 Serial _IEEE-488

Address (0-30): 0
```

Enter an address number for the machine. Set the controller DNA Engine's address to 0. Give the other machines in the network any number from 1 to 30. Press «Cancel» to delete a number and enter a new one.

Running a Protocol on Networked Machines

To run a protocol on networked machines, use the controller to select a protocol (see "Choosing a Stored Protocol to Run," chapter 5), then press «Proceed». The network screen for the controller will be displayed:

```
Run: 2-STEP   on  *


Block: Local (96V)
```

The protocol will be identified on the top line (2-STEP in the example above). The single asterisk in the upper right-hand corner indicates that a single-block Alpha unit is loaded into the controller. "Local" indicates that this is the controller's network screen, and "96V" indicates the type of Alpha unit loaded into the controller.

If a dual-block Alpha unit is loaded into the controller, press «Proceed» until the letter designating the desired block is displayed after the asterisk in the upper right-hand corner of the screen. The letter A designates the right-hand block, and the letter B the left-hand one (e.g., *A, *B).

To run the protocol on the controller's block, press «Proceed». One or more screens for setting up the protocol's temperature control method will be displayed (see "Running a Protocol," chapter 5). When these screens have been worked through, the protocol will begin running.

To run the protocol on a different machine's block, press «Block» until the screen identifies the desired block (22 in the example below):

```
Run: 2-STEP   on  22


Block: Remote 60V
```

The machine's address number will be identified in the upper right-hand corner of the screen. "Remote" indicates that this is the network screen for a slave unit, and "60V" indicates the type of Alpha unit that has been selected. The status light for the selected block will flash on the control panel of the machine holding the block.

If the right or left block of a dual-block Alpha unit has been selected, an A or a B will appear after the address number (e.g., 7-3A for the right-hand block in a dual-block Alpha unit loaded into quadrant 3 of a DNA Engine Tetrad at address 7. See figure 12-1 for a diagram of the layout of quadrants in the Tetrad™ cycler).

Press «Cancel» at any time to abandon the selection process and display the Main Menu again.

When the desired block is identified in the LCD window, press «Proceed». When you have worked through the screens that set up the protocol's temperature control method, the protocol will begin running.

The protocol's runtime screen will appear on the **controller's** LCD window. The LCD window of the machine holding the active block will continue to show whatever screen it was displaying before the protocol began running.

Press «Block» to display the Main Menu on the controller again. Repeat the procedure to run protocols on other networked blocks. Press «Block» on the controller to progressively display the runtime screens of all networked blocks that are running protocols.

While protocols are running, other DNA Engine functions (Edit, Enter, Files, etc.) can be accessed on any individual machine in the network, from that machine's front panel.

Networking Machines with a Computer

Networked machines may be conveniently controlled by a computer using DNA Engine Driver software, which allows you to

- Read and run protocols on any networked machine
- Monitor block temperatures on a real-time graphical display
- Save runtime data to the hard drive for later viewing in spreadsheet or graphical format
- Verify results and keep records of runs

Contact MJ Research or an authorized distributor if you do not already have a copy of this software. A complete manual is provided with the software. Also available from MJ Research is a list of ASCII commands used to communicate with DNA Engines and DNA Engine Tetrads, which

may be used to write your own driver software.

A computer-controlled network can be connected via IEEE-488 cables or RS-232 (serial) cables. In either case, the computer acts as the controller of the network and uses the driver software to communicate with the networked machines.

Creating a Computer-Controlled IEEE-488 Network

To connect DNA Engines and DNA Engine Tetrads in a computer-controlled IEEE-488 network, the computer must be equipped with an IEEE-488 (GPIB) interface card (contact National Instruments, MJ Research, or an authorized distributor for information about purchasing this card). Connect the machines and assign addresses as described under "Networking Machines Without a Computer" (p. 9-2). Make sure that none of the networked machines have been assigned IEEE-488 address 0, as this will cause communication problems with the computer. Install the MJ Research DNA Engine Driver software on the computer, and use it to control the network.

Creating a Computer-Controlled RS-232 Network

In a computer-controlled RS-232 (serial) network, each machine in the network is connected directly to the controlling computer via a serial port. The number of machines that can be included in such a network thus depends on the number of serial ports available on the controlling computer. Use high-quality serial cables to connect the serial port on each machine (see figs. 2-3 and 12-4) to a serial port on the computer. Use the Setup utilities to configure each machine for serial communications (see "Choosing a Remote Port," chapter 8, and select the serial port and the 9600 baud rate). Install the MJ Research DNA Engine Driver software on the computer and use it to control the network.

10 Maintenance

Cleaning the DNA Engine, *10-2*
- Cleaning the Chassis and Block, *10-2*
- Cleaning the Air Vents, *10-2*
- Cleaning Radioactive or Biohazardous Materials Out of the Block, *10-3*

Changing the Fuses, *10-3*

Cleaning the DNA Engine

Cleaning the Chassis and Block

Clean the outside of the DNA Engine and Alpha unit with a damp, soft cloth or tissue whenever something has been spilled on it or the chassis is dusty. A mild soap solution may be used if needed.

Clean block wells with swabs moistened with water, 95% ethanol, or a 1:100 dilution of bleach in water (see the *Slide Chambers Alpha Unit Operations Manual* for instructions on cleaning the Slide Chambers' slide slots). If using bleach, swab wells with water afterward to remove all traces of bleach. Clean spilled liquids out of the block as soon as possible; dried fluids can be difficult to remove. Do not clean the block with caustic or strongly alkaline solutions (e.g., strong soaps, ammonia, bleach at a higher concentration than specified above). These will damage the block's protective anodized coating, possibly causing electrical shorting.

If you use oil in the block (a practice not recommended by MJ RESEARCH; see "Using Oil to Thermally Couple Sample Vessels to the Block," chapter 4), clean the wells whenever the oil has become discolored or contains particulate matter. Use a swab to determine whether cleaning is needed. Clean the block with 95% ethanol as described above. **Oil buildup must be prevented.** Old oil harbors dirt, which interferes with vessel seating and diminishes thermal coupling of sample vessels to the block.

Caution: Do not pour any cleaning solution into the block's wells and then heat the block, in an attempt to clean it. Severe damage to the block, the heated lid, and the chassis will result.

Cleaning the Air Vents

Clean the air intake and exhaust vents with a soft-bristle brush, a damp cloth, or a vacuum cleaner whenever dust is visible in them. The air intake vents are located on the bottom, lower front edge, and back of the machine; the air exhaust vents are located on both sides (see figs. 2-1, 2-3, and 2-4). If these vents become clogged with dust and debris, airflow to the Alpha unit's heat sink is hampered, causing performance problems related to overheating. The air intake vents are particularly likely to collect dust since their holes are much smaller than those of the air exhaust vents.

✔ **Tip:** To prevent problems with overheating, institute a regular program of checking for dust buildup, particularly for robotics installations.

Cleaning Radioactive or Biohazardous Materials Out of the Block

When cleaning machines that have been running radioactive or biohazardous reactions, consult your institution's radiation safety officer or biosafety officer regarding cleaning methods, monitoring, and disposing of contaminated materials.

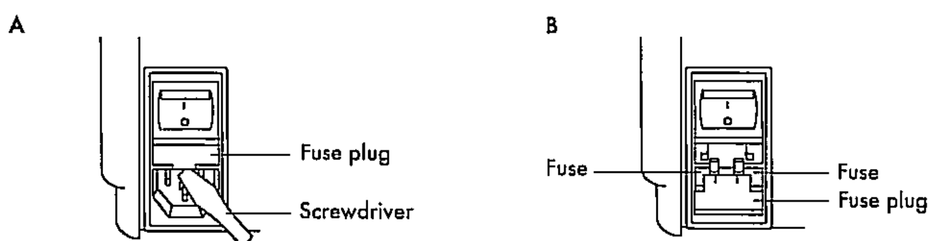
Changing the Fuses

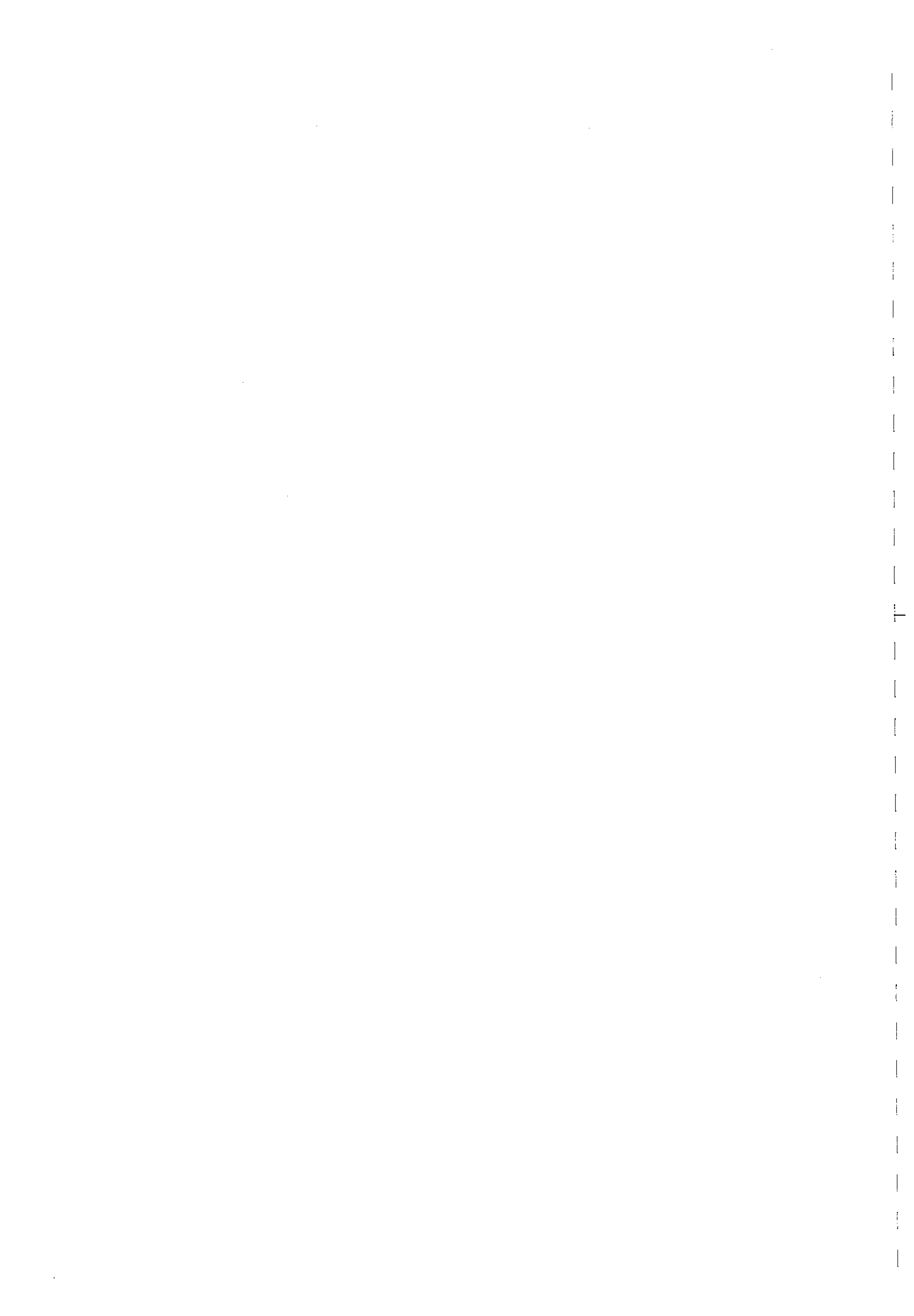
The circuits in the DNA Engine are protected by two fuses (6.3A fast-acting, 5 x 20mm). When a fuse blows, the DNA Engine immediately shuts down and cannot be turned back on. The machine records the event as a power loss, so if a protocol is running when a fuse blows, the machine will resume the run when the fuse is replaced and power restored (see "Resuming a Protocol after a Power Outage," chapter 5).

Warning: The DNA Engine incorporates neutral fusing, which means that live power may still be available inside the unit even when a fuse has blown or been removed. Never open the DNA Engine base. You could receive a serious electrical shock. Opening the base will also void your warranty .

1. Disconnect the power cord from the back of the instrument. Move the power switch to the "0" (off) position.
2. Insert one corner of a small flat-head screwdriver just under the fuse plug (fig. 10-1A), and gently pry the plug loose. Pull the plug straight out as far as it will go, then push it downward to expose the fuses (fig. 10-2B).
3. Remove both fuses and replace them with new ones (it is impossible to visually determine which fuse is blown). You may also test the fuses with an ohmmeter to determine which is defective and replace just that one.
4. Gently press the fuse cover back in place, and reconnect the power cord.

Figure 10-1 A, How to pull out the fuse plug. B, Location of the fuses in the opened plug.





11 Troubleshooting

Error Messages, *11-2*
Problems Related to Protocols, *11-6*
Problems Related to Environmental Conditions, Setup, and Maintenance, *11-8*

Error Messages

Note: The DNA Engine/Tetrad software is highly sensitive with respect to block and heat-sink errors. When such errors messages occur, try restarting the protocol. If the message fails to reappear, proceed as usual.

Error Message	Cause and Result	Action
A/C Power Failed, Cycle XXX Step X, Recovered at XX.X°	Displayed when a machine running a protocol has been turned off, either intentionally or due to a power outage, and then turned on again.	No action is necessary. Protocol resumes running when power is restored. Results may or may not be affected, depending on whether power failed in an early or a late cycle, and whether the power was restored before the sample cooled excessively.
Block Overheated, Return for Service	On machines with software versions below 1.1M, can happen when running calculated-control protocols with target temperatures of 103° 105° C. As the calculated-control algorithm tries to hold samples at such high temperatures, it can heat the block to temperatures in excess of the Alpha unit's maximum allowable temperature (107.5° C), triggering an automatic Alpha unit shutdown.	Determine machine's software version number (see p. 8-9). If number is below 1.1M, contact MJ RESEARCH or your local distributor to request a software version upgrade.
	Sensor malfunction allows base to heat block over its maximum allowable temperature, triggering an automatic Alpha unit shutdown.	Alpha unit and/or base needs servicing. Contact MJ RESEARCH or your local distributor.
Block Sensor Fault, Left Ignored (Center Ignored, Right Ignored)	Left, center, or right temperature sensor in Alpha unit is not working properly, so machine is now ignoring it and relying on remaining sensors to monitor block temperature.	Alpha unit needs servicing soon. Contact MJ RESEARCH or your local distributor.
Block Sensor Fault, Program Terminated	None of the temperature sensors in Alpha unit are working properly, so Alpha unit has been shut down.	Alpha unit and/or base needs servicing. Contact MJ RESEARCH or your local distributor.

Error Message	Cause and Result	Action
Calc Control, Probe Mode Invalid	Runtime error indicating a gradient step is being progammed under Probe Control mode.	Change to Calc control mode.
Gradient Fault, Service Alpha Soon	Displayed at end of run. Indicates gradient was not achieved within 20 secs. of block reaching target.	Alpha unit and/or base needs servicing. Contact MJ Research or your distributor.
Gradient Program, Invalid Alpha Type	Runtime error indicating gradient is being programmed for an Alpha module other than a 96V.	Use 96V Alpha module.
Heated Lid Fault, Program Terminated	Lid sensor failed during preheat, so Alpha unit has been shut down.	Heated lid or base needs servicing. Contact MJ RESEARCH or your local distributor.
HS Overheated, Program Terminated	Machines are not getting enough air, or air being taken in is warmer than 31° C.	Make sure machine gets enough air and that temperature of air being taken in is 31° C or cooler (see p. 3-3). Correct air supply problems and run protocol again. If error message persists, base may need servicing. Contact MJ RESEARCH or your local distributor.
	Machine is running a protocol consisting of many cycles of only a few seconds each. Alpha unit heat sink does not have time to dissipate heat generated by rapid cycling. Eventually its maximum allowable temperature is exceeded, and Alpha unit is shut down.	Contact MJ RESEARCH or your local distributor to discuss protocol.
	Sensor malfunction has allowed base to heat block over its maximum allowable temperature (107.5ŒC), triggering automatic Alpha unit shutdown.	Alpha unit and/or base needs servicing. Contact MJ RESEARCH or your local distributor.
HS Overheating, Check Air Flow	See causes for "HS Overheated, Program Terminated."	See actions for "HS Overheated, Program Terminated."
HS/PS Sensor Fault, Program Terminated	Heat sink sensor in Alpha unit and power supply sensor in base are not working, so Alpha unit has been shut down.	Alpha unit or base needs servicing. Contact MJ RESEARCH or your local distributor.

Error Message	Cause and Result	Action
HS Sensor Fault, Alt Control Mode	Alpha unit heat sink sensor is not functioning properly, so machine has begun estimating heat sink temperature based on other tem-peature sensor readings.	Alpha unit needs servicing soon. Contact MJ RESEARCH or your local distributor.
HS Sensor Fault, Left Ignored	Same as previous	Same as previous
HS Sensor Fault, Right Ignored	Same as previous	Same as previous
Internal Fan Fault, Return for Service	Fan inside base is not working, so Alpha unit has been shut down.	Fan must be repaired. Contact MJ RESEARCH or your local distributor.
Lid Sensor Fault, Lid Was Disabled	Heated lid is giving unexpected temperature readings, so base has turned it off. Protocols can still be run, but reactions may fail be-cause of condensation in vessels.	Alpha unit needs servicing soon. Contact MJ RESEARCH or your local distributor.
L/R Power Imbalance	Thermoelectric units in Alpha unit block are not heating uniformly, so machine begins delivering equal power to all heating ele-ments. Protocols may still be run, but reactions may fail due to un-even block temperatures.	Alpha unit needs servicing soon. Contact MJ RESEARCH or your local distributor.
Memory Is Corrupt!	Rarely seen message indicating that memory has been corrupted by a static shock or other unusual electronic incident.	Base needs servicing. Contact MJ RESEARCH or your local distributor.
No Memory Available!	All available memory has been filled.	Delete unused protocols and folders from memory. Reduce size of stored programs by using GoTo and the Inc and Ext options (see chapter 6).
Probe Sensor Fault, Used Calc Control	Either probe sensor is not working properly or a probe-control pro-tocol was run without probe being installed. Machine has switched temperature control method to calculated control.	Make sure probe is installed when running probe-control programs. If error message persists, replace probe.

Error Message	Cause and Result	Action
PS Overheated, Program Terminated	Machine is not getting enough air, or air being taken in is warmer than 31° C.	Make sure that machine is getting enough air and that temperature of air being taken in is 31° C or cooler (see p. 3-3). Correct any air supply problems and run protocol again. If error message persists, base may need servicing. Contact MJ Research or your local distributor.
	Machine is running a protocol consisting of many cycles of only a few seconds each. Alpha unit heat sink does not have time to dissipate heat generated by rapid cycling. Eventually its maximum allowable temperature is exceeded, and Alpha unit is shut down.	Contact MJ Research or your local distributor to discuss protocol.
PS Overheating, Check Air Flow	See causes for "PS Overheating, Program Terminated."	See actions for "PS Overheating, Program Terminated."
PS Sensor Fault, Temp Ignored	Power supply sensor is not working properly. Protocols can still be run, but machine should be serviced soon too repair sensor.	Base needs servicing. Contact MJ Research or your local distributor.
Slow Block Cycling, Service Alpha Soon	Alpha unit block has not reached target temperature within expected time. Unit will begin beeping and will continue to beep until target temperature is reached, protocol is manually progressed to its next step (see p. 5-8), or protocol run is halted. Problem often results from machine not getting enough air, or taking air that is warmer than 31° C.	Make sure machine is getting enough air and that temperature of air being taken in is 31° C or cooler (see p. 3-3). Correct any air supply problems and run protocol again. If error message persists, software upgrade may be needed or base may need servicing. Contact MJ Research or your local distributor.
Slow Lid Cycling, Service Alpha Soon	Alpha unit lid has not reached target temperature within expected time. Unit will begin beeping and will continue to beep until target temperature is reached, protocol is manually progressed to its next step (see p. 5-8), or protocol run is halted.	Alpha unit lid needs servicing soon. Contact MJ Research or your local distributor.
Unit Failure/ Unit X Failure (Tetrad)	The self-test has failed because of a problem with the base or the Alpha unit.	Contact MJ Research or your local distributor.

Problems Related to Protocols

Following is a general description of some common problems related to the protocols and reaction components in sequencing and amplification applications. For a more detailed discussion of protocols and reactions, see *Current Protocols in Molecular Biology* (F. Ausubel et al., eds., John Wiley & Sons), specifically chapters 7 (DNA sequencing), 14 (*in-situ* hybridization and immunology), and 15 (polymerase chain reaction).

Error Message	Cause and Result	Action
Reaction is working but broad low molecular weight band is seen in gels.	"Primer-dimer" material often produces a broad band in the <100bp region of gels.	If obtaining appropriate reaction product/s, no need to change anything.
		Minimize "primer-dimer" production by designing primers with no 3' self-complementarity.
		Reoptimize magnesium concentration and annealing temperature to maximize desired product and minimize "primer-dimers."
Reaction working but unexpected extra products or smear is seen.	Nonspecific hybridization occurring during setup.	Program a hot start into the protocol.
	Reaction component concentration too high or too low.	Check concentratons of components. May need to reoptimize magnesium concentration.
	Annealing temperature too low.	Reoptimize annealing temperature.
	Protocol contains a wrong value.	Use List utility to check protocol's temperature control method, temperatures, and times.
	Template not of sufficient purity.	Check extraction and purification protocols. Add additional purification steps if necessary.
	Multiple templates or host DNA in sequencing reactions.	Check nucleic acid preparations by gel electrophoresis.

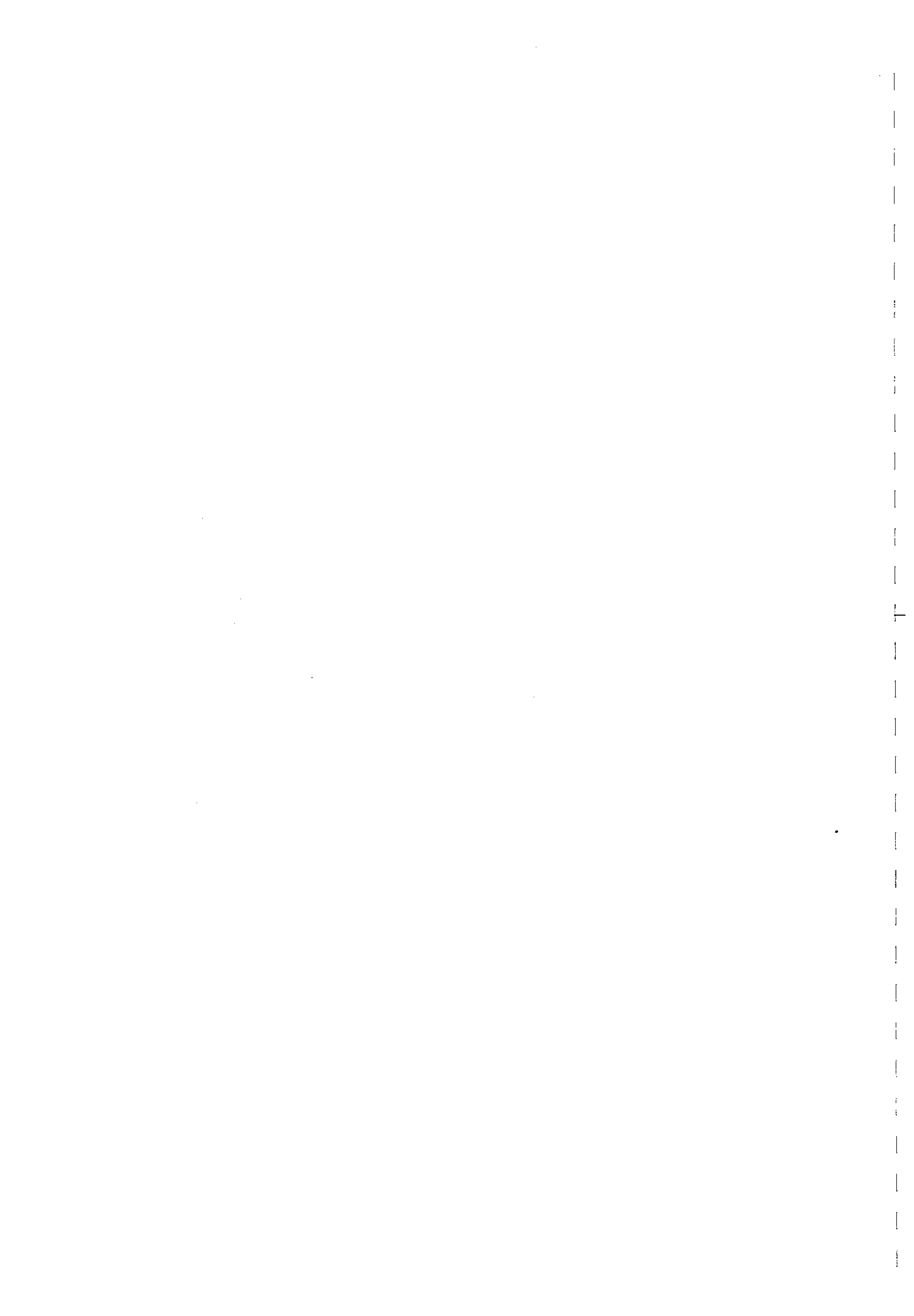
Problem	Cause	Action
No reaction products obtained.	Wrong protocol used.	Re-run reaction using correct protocol.
	Protocol contains a wrong value.	Use List utility to check protocol's temperature control method, temperatures, and times.
	Reaction component omitted from mixture.	Check reaction assembly protocol, ensuring that mixture contains appropriate components in correct concentrations.
	Denaturation temperature too low.	Use ≥92° C for denaturation.
	Annealing temperature too high for primers.	Check for appropriate annealing temperatures of primers, using available computer programs or empirical testing.
	Wrong temperature control method used.	Use List utility to check temperature control method for protocol; change if needed.
	Probe failed, causing machine to run protocol under calculated control.	Check screen for probe failure error message. Probe may need servicing or replacing. Call MJ RESEARCH or your local distributor.
	Probe not filled with correct amount of oil.	Fill probe tube with correct amount of oil (see p. 4-11).
	Reaction mix contains an inhibitor (e.g., heme from blood).	"Spike" a complete reaction mix with a control template and primer set.
	Reaction vessels not making good thermal contact with sample block.	Use only high-quality tubes/plates that fit block snugly. Ensure that wells are free of foreign materials that would interfere with tube/plate seating.

Problems Related to Environmental Conditions, Setup, and Maintenance

Problem	Cause	Action
Frequent shutdowns due to overheating. Frequent "Slow Block Cycling," "HS Overheat," and "HS Overheating" error messages.	Machine is not receiving enough air.	Make sure air intake vents are not obstructed by dust, debris, or paper. Remove light collections of dust and debris with damp cloth. Vacuum out heavy collections. Remove any papers placed under the machine. Position machine at least 10cm from vertical surfaces.
	Air flowing into intake vents is not ≤31° C.	Check temperature of air entering air intake vents, following procedure on p. 3-3. If higher than 31° C, use table 3-1 to troubleshoot and remove cause/s.
Dust and debris clogging up air intake vents.	Failure to regularly check for buildup.	Remove light collections with damp cloth. Vacuum out heavy collections.

Part II

The DNA Engine Tetrad®
Peltier Thermal Cycler



12 Information Specific to the DNA Engine Tetrad

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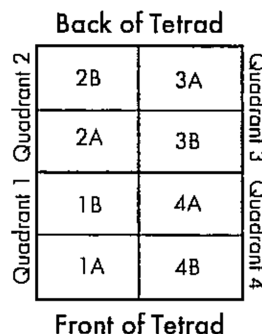
Meet the DNA Engine Tetrad® Thermal Cycler

The DNA Engine Tetrad thermal cycler harnesses four DNA Engine® cyclers in a single compact machine. Four Alpha™ units of any type can be loaded into the DNA Engine Tetrad base, one unit into each "quadrant" of the base. The same factory-installed and custom protocols can be run on the DNA Engine Tetrad as on the DNA Engine. Programs can be run independently on any one Alpha unit or simultaneously on all of them.

The DNA Engine Tetrad's control panel is nearly identical to that of the DNA Engine. Additional features include a «Power» key and an array of lights indicating the selection status of the blocks in the base's quadrants. There is an A and a B light for each quadrant since each Alpha unit can contain up to two blocks. The arrangement of the status indicator lights matches the arrangement of the quadrants in the base when the DNA Engine Tetrad is viewed from above (fig. 12-1).

Figure 12-1 Layout of quadrants.



The DNA Engine Tetrad's power supply has been placed in a separate housing that is attached by cables to the base, which keeps the base compact and makes it easier to use in robotics installations. The power supply uses MOSFET transistors to efficiently convert AC input power into the high-current, low-voltage DC power that the Tetrad requires (see appendix D for a short explanation of this technology).

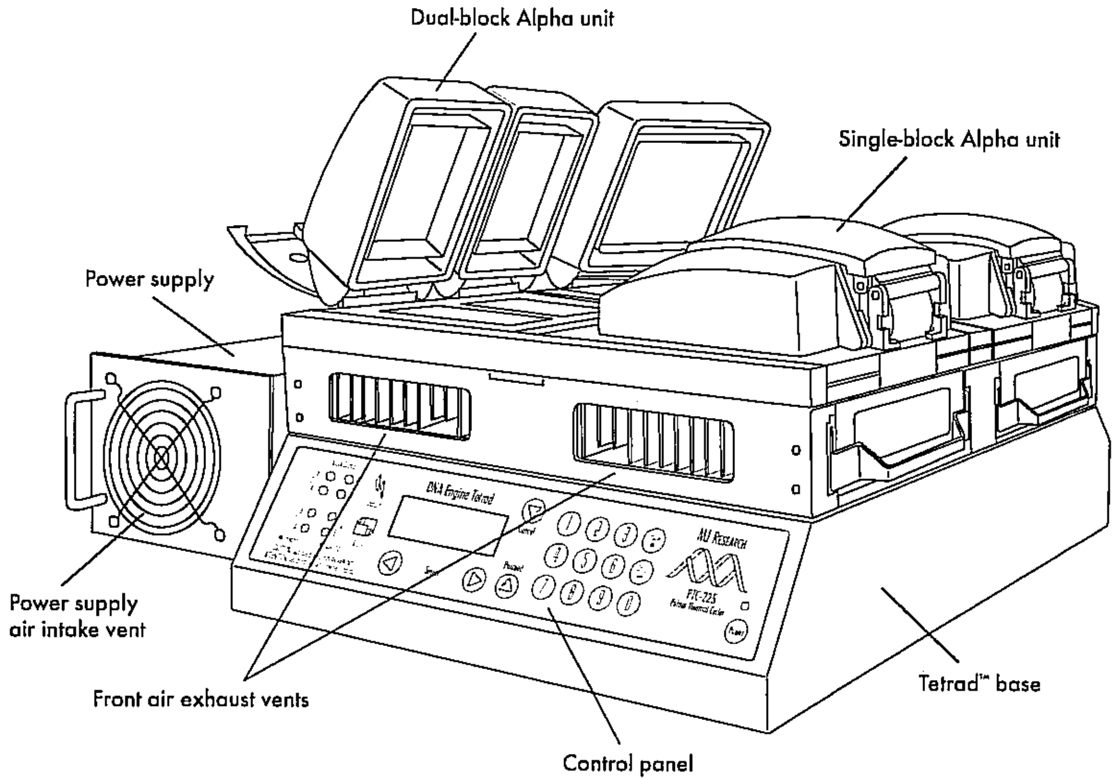
Up to 15 DNA Engine Tetrads and DNA Engines can be networked together, in any combination, but a DNA Engine or a computer must serve as the controller unit for the network. Networked DNA Engine Tetrads operate like networked DNA Engines, with a few minor exceptions (explained below). The Moto Alpha remote-controlled heated lid may also be used with networked DNA Engine Tetrads if the controller is a computer.

Specifications

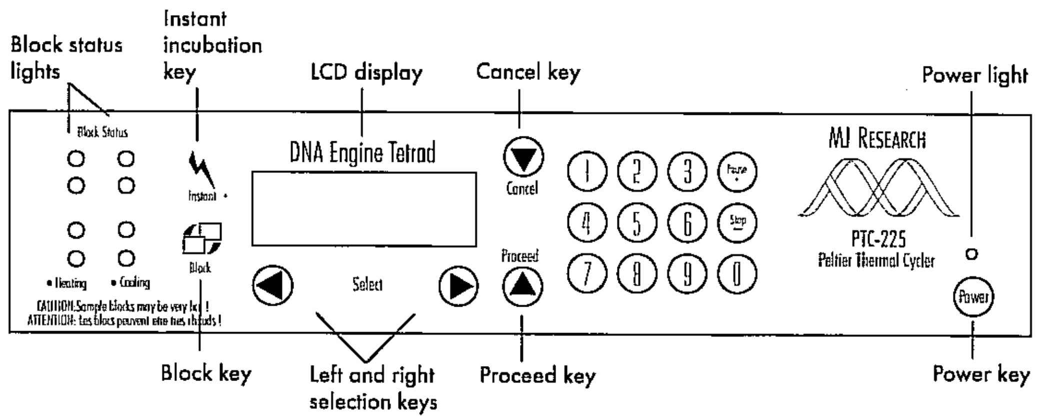
The DNA Engine Tetrad's specifications are identical to those of the DNA Engine except for weight and size (see chapter 2).

Layout

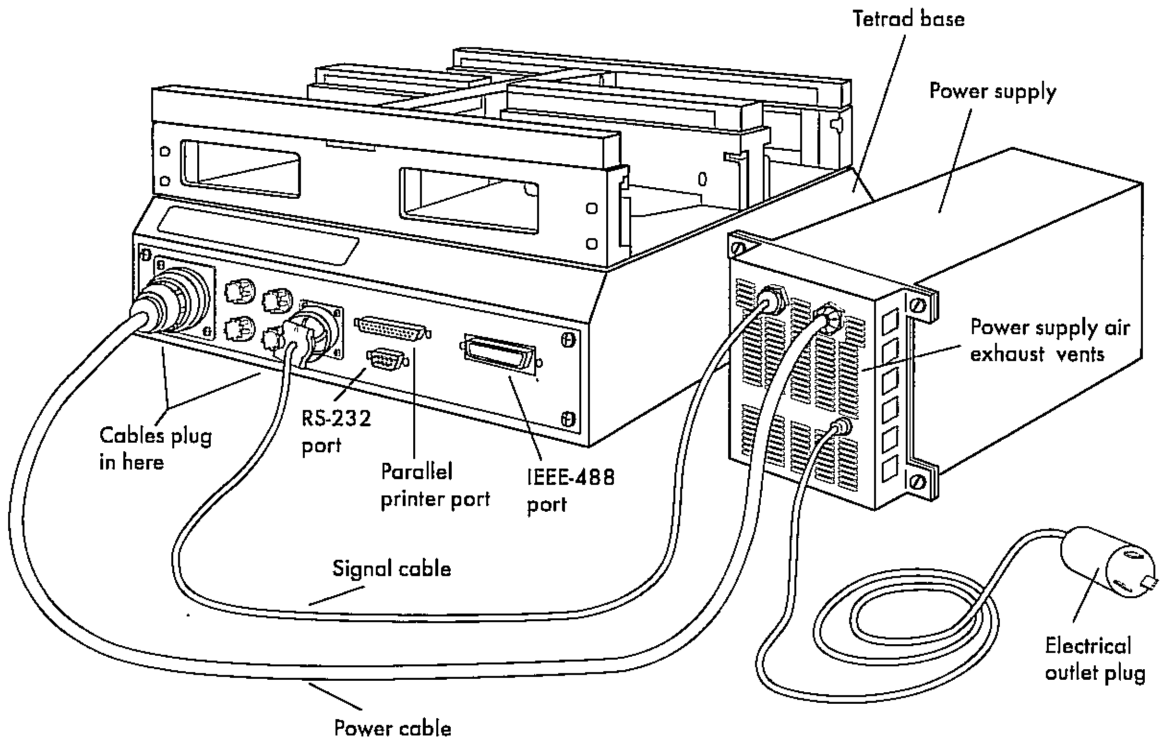
Front View
(Figure 12-2)



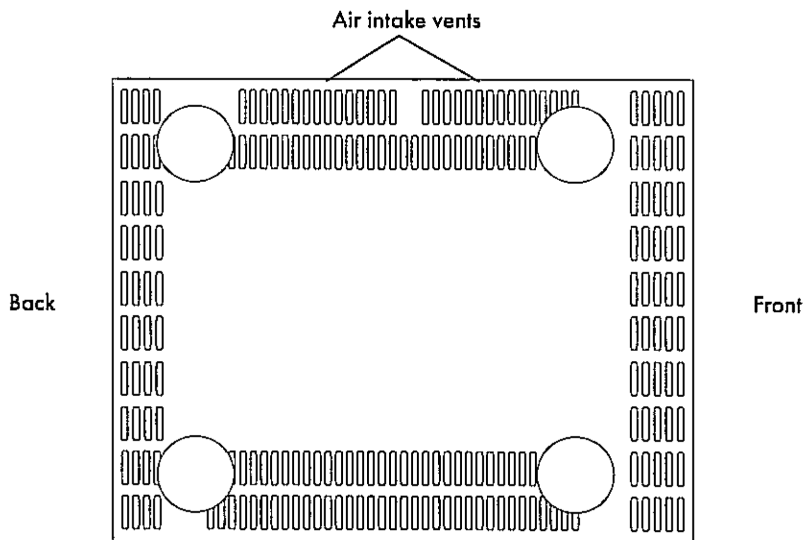
Control Panel
(Figure 12-3)



Back View
(Figure 12-4; Alpha units removed)



Bottom View
(Figure 12-5)



Unpacking and Installing the DNA Engine Tetrad

Packing Checklist

After unpacking the DNA Engine Tetrad, check to see that you have received the following:

- DNA Engine Tetrad base
- Four Alpha units
- Power supply unit
- 220V power cord set (inside power supply box)
- Four spare fuses
- *PTC-200 DNA Engine & PTC-225 DNA Engine Operations Manual* (this document)

If any of these components are missing or damaged, contact MJ RESEARCH or the authorized distributor from whom you purchased the DNA Engine Tetrad to obtain a replacement. Please save the original packing materials in case you need to return the Tetrad for service. See appendix C for shipping instructions.

Installation

The DNA Engine Tetrad requires minimal assembly: installing the Alpha units (see chapter 4) and connecting the signal and power cables to the back panel of the base.

Connecting the Cables

The signal and power cables running from the DNA Engine Tetrad's power supply (fig. 12-4) must be connected to the base, and the power supply must be plugged into an electrical outlet.

Warning: Disconnect the power supply from the wall outlet *before* connecting or disconnecting the signal or power cable. The power supply is capable of generating high-current DC power. High-current circuits should never be connected or disconnected when electrical power is present; electrical arcs may result, which can damage the power supply and pose a hazard of electrical shock.

The signal and power cables terminate in twist-lock plugs. To screw a plug in, first make sure the power supply is not plugged into an outlet. Press the plug into the socket, and twist it to the right. A snap will be felt when the plug completely seats in the socket; a faint click is also audible when the plug to the power cable fully seats.

The signal and power cables are joined at their power supply end by a single connector that plugs into the power supply. This connector is housed in a protective metal cage screwed onto the back of the power supply.

Note: Do not open the protective housing; there are no user-serviceable parts within.

Power Supply Requirements

The DNA Engine Tetrad requires 10A@200–240VAC, 50/60 Hz. The unit is not designed to operate on the ordinary 100V–120V household power commonly supplied in North America and Japan. The DNA Engine Tetrad can use current in the specified range without adjustment, so there is no voltage-setting switch.

Environmental Requirements

The requirements for safe operation of the DNA Engine Tetrad are the same as those of the DNA Engine (see chapter 3).

Air Supply Requirements

The air supply requirements for the DNA Engine Tetrad are the same as those of the DNA Engine (see chapter 3). .

Robotics Installation Requirements

Robotics installations of DNA Engine Tetrads require special attention to airflow and air temperature. Typically in these installations, DNA Engine Tetrads and other thermal cyclers are crowded into a small area, along with other heat-generating equipment. Overheating can quickly occur when many of these machines are operating at once, unless preventive measures are taken.

Follow the procedures described in chapter 3 to ensure adequate airflow and an air intake temperature of 31°C or cooler. Air intake temperature must be verified by measurement. If none of the troubleshooting procedures outlined in chapter 3 are sufficient to ensure cool-enough air, install ductwork to the front and rear air exhaust vents, to remove exhausted air. Attach ductwork using the screws to the right and left

of the air exhaust vents on the front and back of the DNA Engine Tetrad (fig. 12-2). These screws may be replaced with longer ones, as long as they are M4 x 0.7 screws.

Operating the DNA Engine Tetrad

Turning the DNA Engine Tetrad On

First, **make sure Alpha units are installed in all four quadrants.** An interlock switch prevents the DNA Engine Tetrad from operating if fewer than four Alpha units are installed.

Turn the DNA Engine Tetrad on with the «Power» key located at the lower right of the control panel.

Operating Alpha Units

Alpha unit installation, removal, and general operation are the same as for the DNA Engine (see chapter 4).

Running Protocols on the DNA Engine Tetrad

Running Separate Protocols by Block

Choose a protocol (see chapter 5). A screen identifying the protocol (2-STEP in the examples below), the Alpha unit type (96V), and the quadrant number of the block the protocol will run on (1) will be displayed:

```
Run: 2-STEP    on  1


Block: 96V
```

If a dual-block Alpha unit is present in the identified quadrant, an A or a B will appear after the quadrant number (e.g., 1A).

Protocols are initially assigned to the first block available in the DNA Engine Tetrad, in numerical order of quadrants. To run the protocol on a different block, press «Block» until the quadrant number of the desired block is displayed in the screen's upper right-hand corner. The status light of the selected block will flash. When a single-block Alpha unit is selected, only the A light flashes. When a dual-block Alpha unit is selected, either the A or the B light flashes, depending on the block chosen.

When the desired block has been selected, press «Proceed» to run the protocol.

Note: To run different protocols concurrently, press «Block» to return to the main screen, and select a new protocol and a new block as described in this section. Any protocols already initiated will continue to run.

Running the Same Protocol on All Blocks

If all the Alpha units installed in the DNA Engine Tetrad have the same type of block (i.e., each has the same number of wells, or four Slide Chambers Alpha unit are loaded) **and** no protocols are currently running on any of them, the same protocol (including an instant incubation) may be run on all of the blocks at once.

To do this, select a protocol (or press «Instant» to run an instant incubation), then press «Block» until *All* appears in the upper right-hand corner of the displayed screen:

```
Run: 2-STEP   on ALL


Block: 96V
```

The status indicator lights for all of the sample blocks will be flashing green. Press «Proceed» to run the protocol on all the blocks simultaneously.

Running an Instant Incubation

Instant incubations are run in the same way as for the DNA Engine (see chapter 5). The incubation is initially assigned to the first available block in numerical order of quadrants; press «Block» to assign it to a different block, or to all of them (if the same type block is present in all four quadrants).

Cancelling Protocols

Press «Block» to select the block that is running the protocol to be cancelled, then press «Cancel». A cancellation confirmation screen will be displayed:

```
Run: 2-STEP    on 2B

STOP 2-STEP on 2B?
       Yes  _NO
```

Select *Yes*, then press «Proceed». A screen announcing the cancellation and citing the total time for the run will be displayed:

```
Run: 2-STEP    on 2B
  PROGRAM CANCELED

Total time:     1:10
```

Programming the DNA Engine Tetrad

The DNA Engine Tetrad is programmed in the same way as the DNA Engine (see chapter 6).

Networking the DNA Engine Tetrad

Any combination of DNA Engine Tetrads and DNA Engines can be networked, as long as the 15-machine limit is not exceeded and a computer or a DNA Engine is included to serve as the controller (the DNA Engine Tetrad cannot serve as controller). See chapter 9 for information on networking machines.

Software Required for Networking

A DNA Engine must have software version 1.1J or later in order to control a network that includes one or more DNA Engine Tetrads. To identify the software version loaded into a DNA Engine, see "Determining the Software Version Number," chapter 8.

Operating Networked DNA Engine Tetrads

Networked DNA Engine Tetrads operate the same as networked DNA Engines. Each block of a networked DNA Engine Tetrad is designated by the machine's IEEE-488 address, followed by the block's quadrant number and an A or a B if a dual block is involved. For example, the designation for a single-block Alpha unit present in quadrant 1 of machine 17 is 17-1. The designation for block B in a dual-block Alpha unit present in quadrant 2 of the same machine is 17-2B.

Using the Utilities for the DNA Engine Tetrad

The DNA Engine Tetrad has the same utilities as the DNA Engine (see chapter 8).

Troubleshooting the DNA Engine Tetrad

The Tetrad is subject to the same problems with operations, protocols, and reaction results as the DNA Engine (see chapter 11).

Maintaining the DNA Engine Tetrad

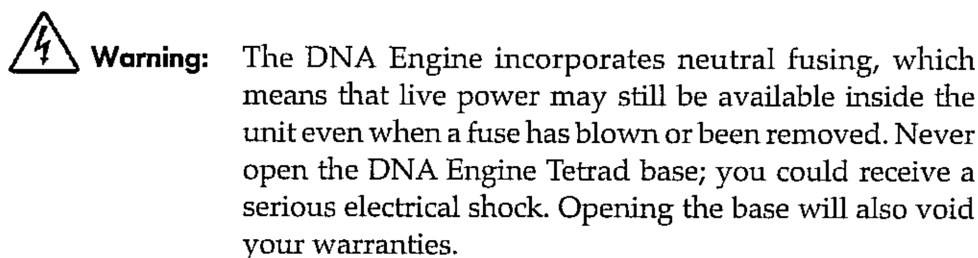
Cleaning

Clean the DNA Engine Tetrad according to the instructions in chapter 10 for cleaning the DNA Engine.

Changing the Fuses

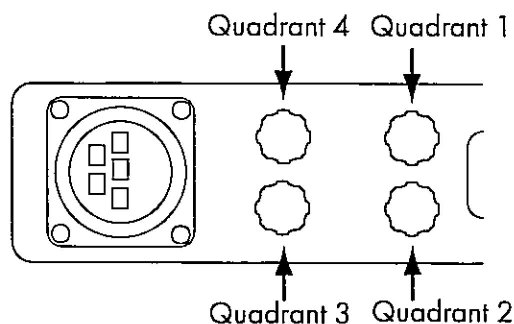
Each quadrant of the Tetrad is protected by a fuse mounted in a plug on the back panel of the base (fig. 12-6). Blown fuses may be replaced by any 15A fast-acting fuse (0.25" x 1.25"3AB or 3ABC).

To replace a fuse, first **unplug the power supply from the wall.** Twist the fuse cap a quarter turn to the left and pull out the fuse plug. Replace the blown fuse. Press the plug back into its socket, and twist it a quarter turn to the right to seat it. The plug is spring-loaded, so firm pressure may be required.



Warning: The DNA Engine incorporates neutral fusing, which means that live power may still be available inside the unit even when a fuse has blown or been removed. Never open the DNA Engine Tetrad base; you could receive a serious electrical shock. Opening the base will also void your warranties.

Figure 12-6 Quadrant assignments for fuses.



Part III

Accessories

13 The Remote Alpha Dock™ System

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Installation, *13-5*
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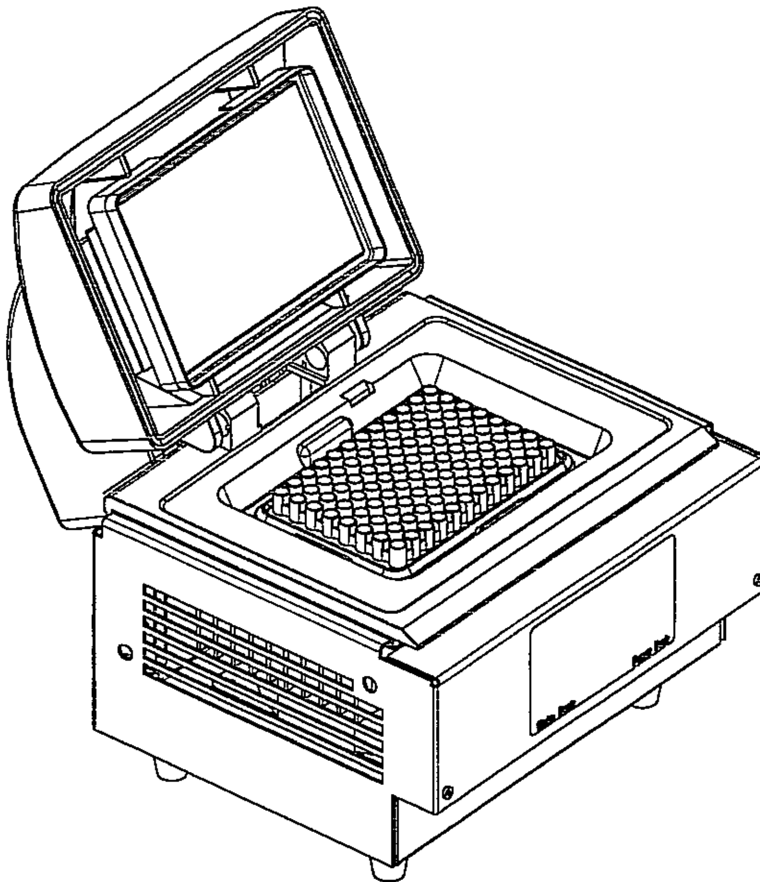
About the Remote Alpha Dock System

The Remote Alpha Dock system is designed to add flexibility to the installation and operation of the MJ Research® DNA Engine® and DNA Engine Tetrad® cycler. The system allows Alpha™ units to be placed at a distance from the PTC-200 or PTC-225 base, enabling more efficient use of space and facilitating robotic operation. The basic system, the RAD-0200, comprises a Dock Connector, which mounts in the base; and a Remote Alpha Dock, into which the Alpha units are mounted. The RAD-0201 contains, in addition, a fan power supply and three "daisy chain" (series) cables, allowing the cooling fans for up to four Remote Docks to run off a single AC outlet. Additionally, the fan power supply along with the cables to run up to four Remote Alpha Dock fans from a single thermal cycler base can be purchased separately, as RPS-0200.

Figure 13-1 The Remote Alpha Dock with Alpha unit mounted



Packing checklist

- One Dock Connector
- One Remote Alpha Dock
- One multi-pin power cable
- One multi-pin data cable
- One fan power supply (RAD-0201 only)
- One wall-plug power cord (RAD-0201 only)
- Three round-jack power cords (RAD-0201 only)

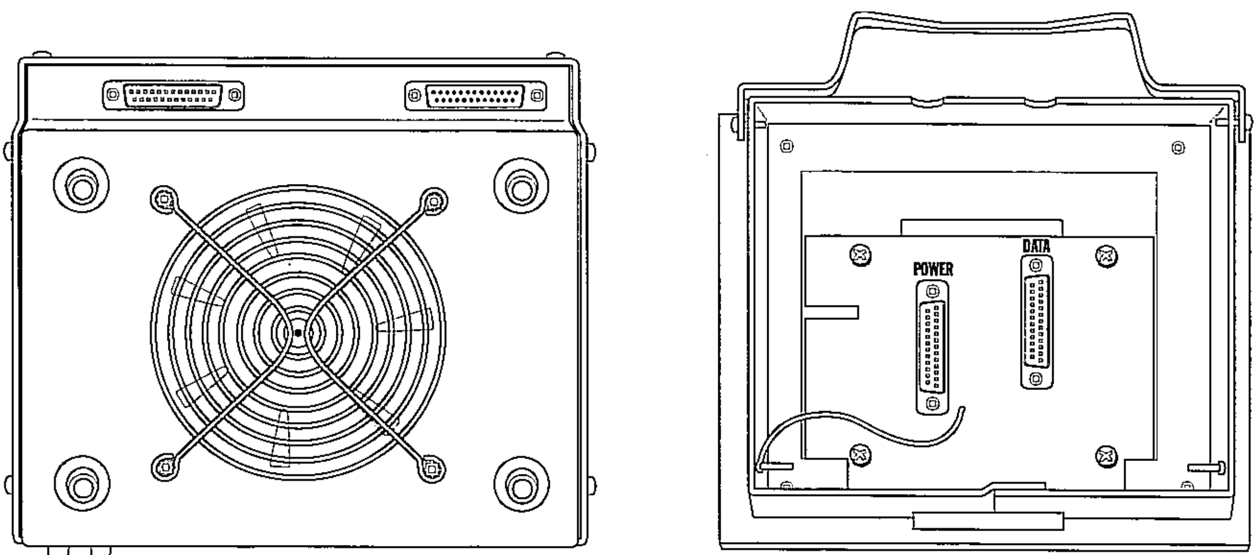
Requirements

Environment

The Remote Alpha Dock System allows for custom installations. The following placement configurations are recommended by MJ Research.

- Remote Docks with mounted Alpha units may be configured in any horizontal orientation or array as long as a minimum side clearance of 10 cm is maintained between the Remote Dock and any wall, bulkhead, or adjacent Remote Dock unit (this is identical to the PTC-200/ PTC-225 base requirement). Requirements for motorized lid operation or for loading or unloading plates may dictate additional clearances.
- Remote Docks with mounted Alpha units may be stacked vertically as long as a minimum bottom clearance is maintained that would be

Figure 13-2 Remote Alpha Dock and Dock Connector, bottom view.



no less than that resulting from the unit being placed on a solid horizontal platform. A minimum top clearance is also required to allow access to and operation of the Alpha unit lid.

- Remote Docks can be flush-mounted (i.e., with the feet removed) to facilitate robotic operation, as long as the airway beneath the unit is equivalent to the airway the unit would have with the feet attached. Usually a hole will need to be cut to allow air to flow to the cooling fan. Figure 13-5 is a template for flush-mounting the Remote Dock.

Power Supply

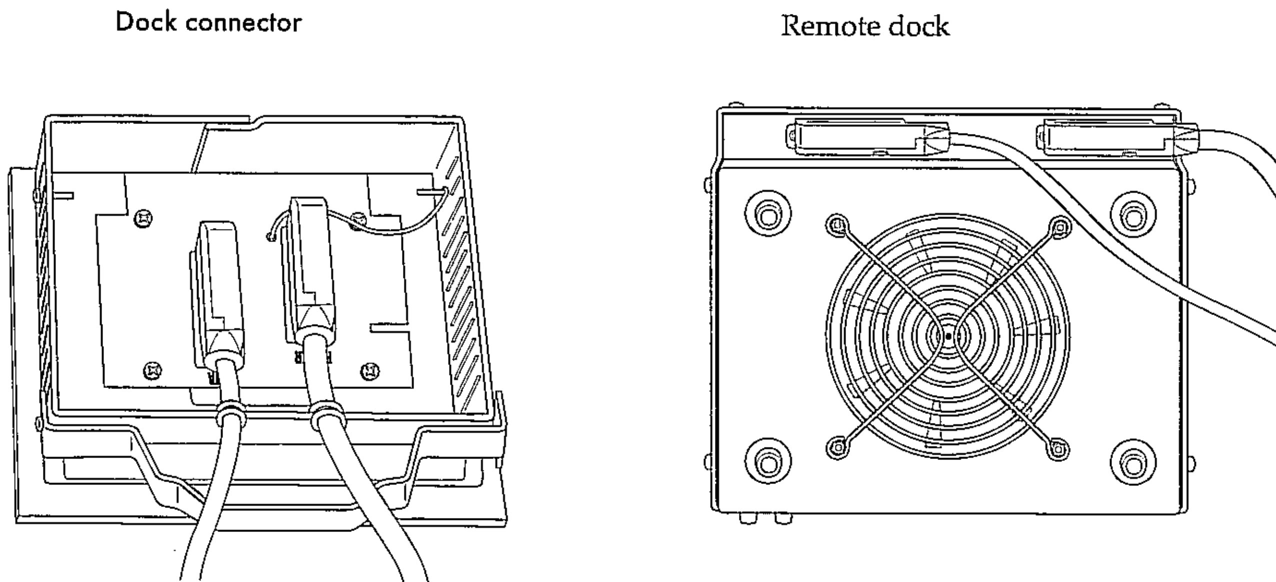
- The Alpha unit mounted in each Remote Dock is powered from the PTC-200 or PTC-225 base.
- The Remote Dock's fan is powered externally, and a power supply is provided that requires power from 90-250 VAC and 47 to 63 Hz, with a grounded outlet.

Air Supply

- Alpha units being operated in the remote configuration have no operating constraints that do not also apply to normal operations in the PTC-200 or PTC-225.

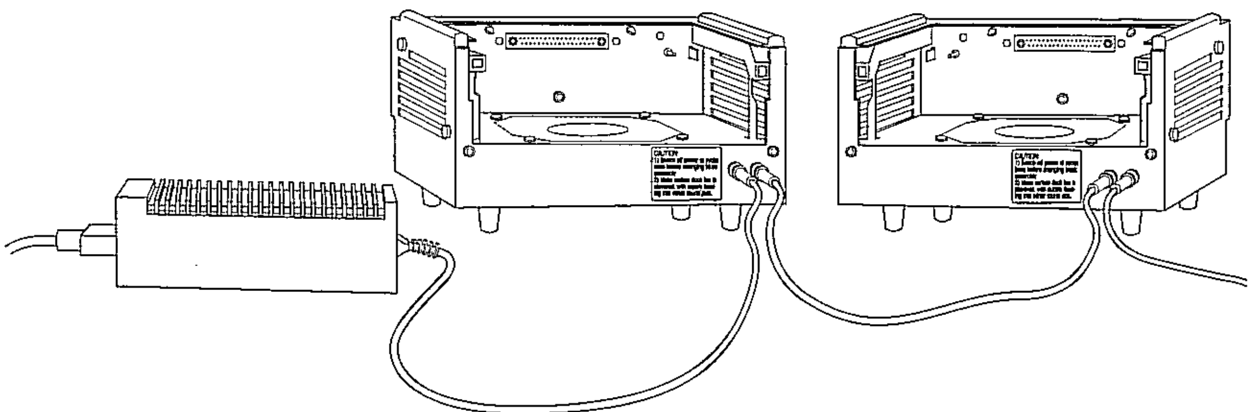
Installation

Figure 13-3 Attachment of power and data cables



- Turn the Dock Connector upside down, so that the green circuit board is visible. Check that the ground lead remains attached at both ends (fig. 13-1). Note the two female multi-pin sockets, one labeled "DATA," and the other "POWER."
- Connect the multi-pin power cable's male end to the female socket labeled "POWER" and slide the latch to lock the pins in place.
- Both of the data cable's multi-pin connectors are male: one is labeled "CONNECTOR DATA" and the other "DOCK DATA." Attach the "CONNECTOR DATA" end to the female connector labeled "DATA" on the circuit board and slide the latch to lock the pins in place.
- Press both cables firmly into the two strain relief holes on the Dock Connector's front side (fig. 13-2).
- Turn the Remote Dock upside down. You will see a male multi-pin connector labeled "Power Port" and a female multi-pin connector labeled "Data Port" (fig. 13-3).
- Connect the multi-pin power cable's female end to the connector labeled "Power Port" and slide the latch to lock the pins in place.
- Attach the data cable's "DOCK DATA" end to the connector labeled "Data Port" and slide the latch to lock the pins in place.
- Turn both units back over.
- The Dock Connector mounts in the base in the same manner as a regular Alpha unit (see pp. 4-4 – 4-6).
- Attach the wall-plug power cord to the fan power supply.

Figure 13-4 Fan power supply connected in series



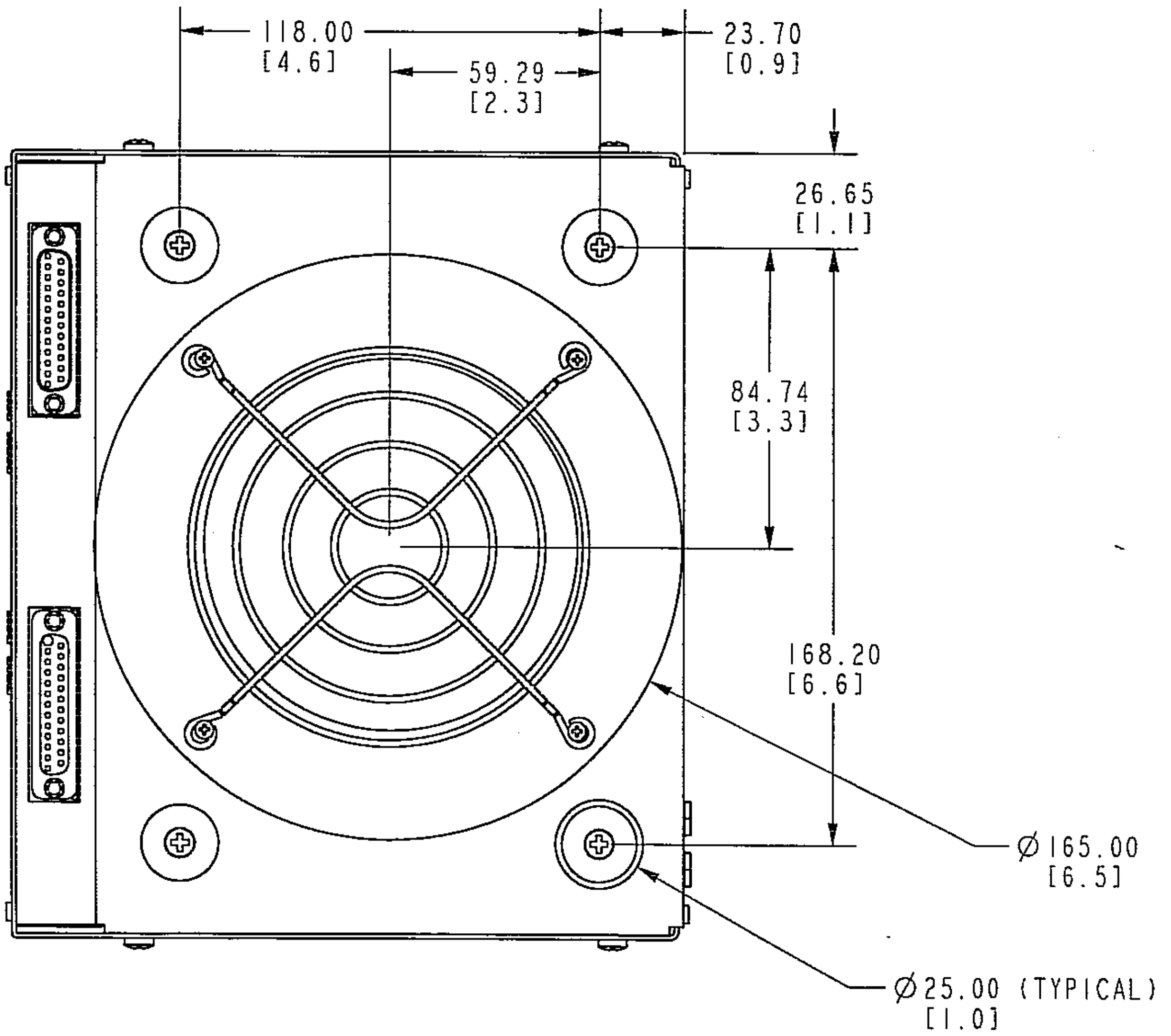
- Attach the fan power supply's round jack to either of the round connectors on the back of the Remote Dock (fig. 13-4).
- The fan power supply produces sufficient current such that up to three additional fans can be "daisy chained" to the initial Remote Dock via the round-jack power cords supplied. Attach one end of the cord to the initial unit's free round connector. Attach the other end to either of the round connectors on the back of the next Remote Dock in the series, and so on (fig. 13-4).
- When the RAD system has been completely set up, connect the fan power supply's wall plug to a power source.

Operation

The remote system is transparent to the base unit; i.e., the Dock Connector allows the base to control the Alpha unit in the Remote Dock as if it were in the standard configuration.

IMPORTANT: Turn the base unit's power off when changing the type of sample block you are using. Turning the power off resets the base, allowing it to recognize the new block. If not reset, the base unit assumes that the previous type of block is installed, resulting in error messages and procedural faults.

Figure 13-5 Flush-mounting template



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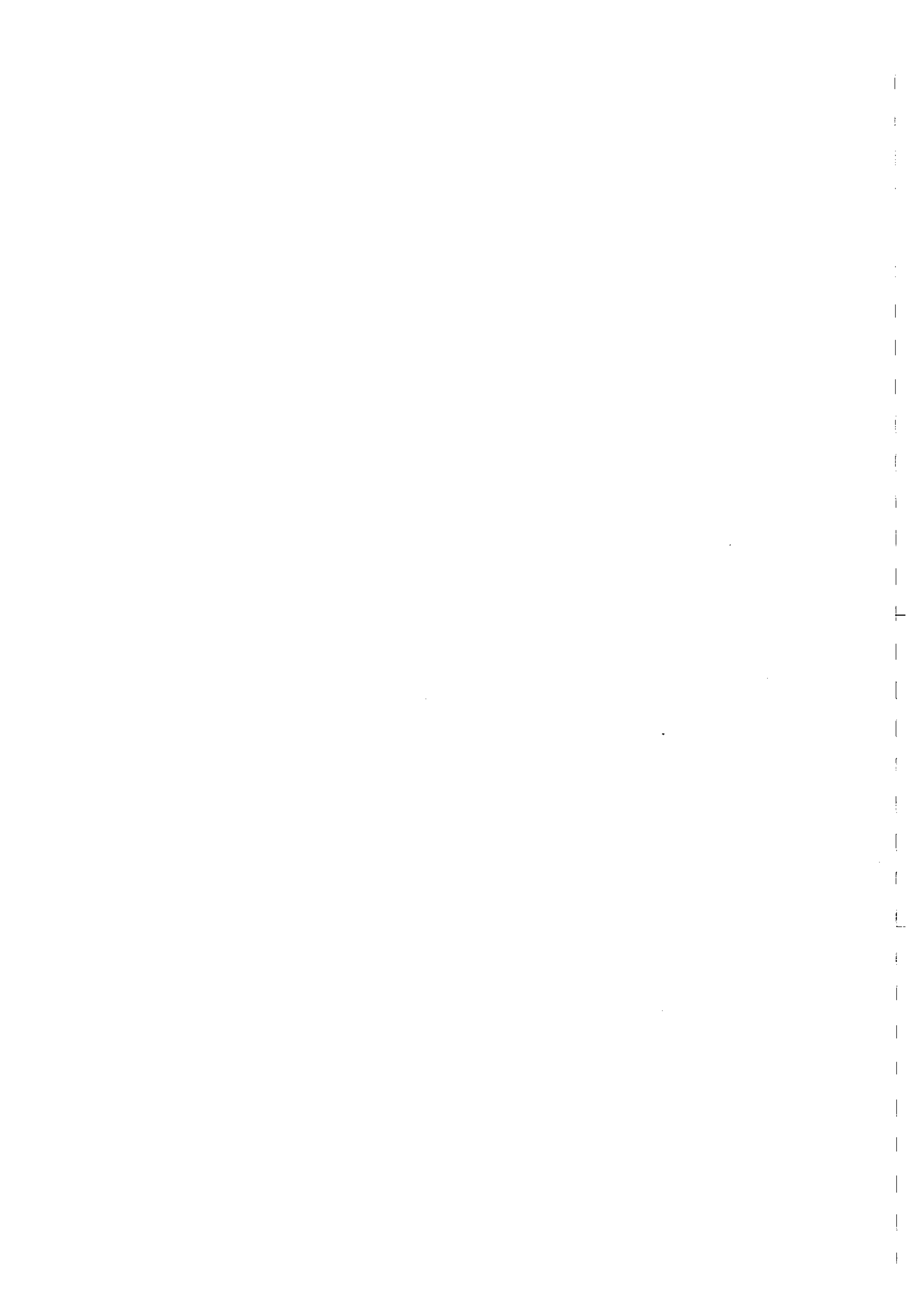


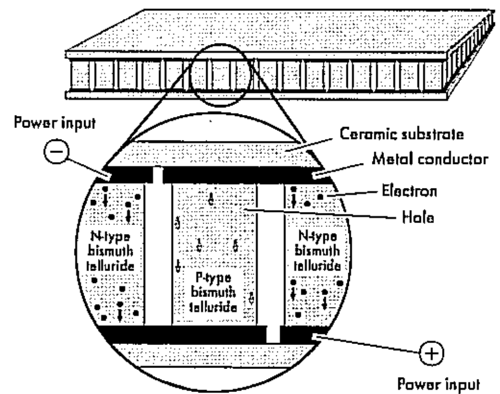
Appendix A

How a Peltier Heat Pump Works

The functional heart of every DNA Engine is a high-performance Peltier-effect heat pump (also known as a "thermoelectric module"). This solid-state device is manufactured to withstand the thermal stresses associated with rapidly cycling temperatures.

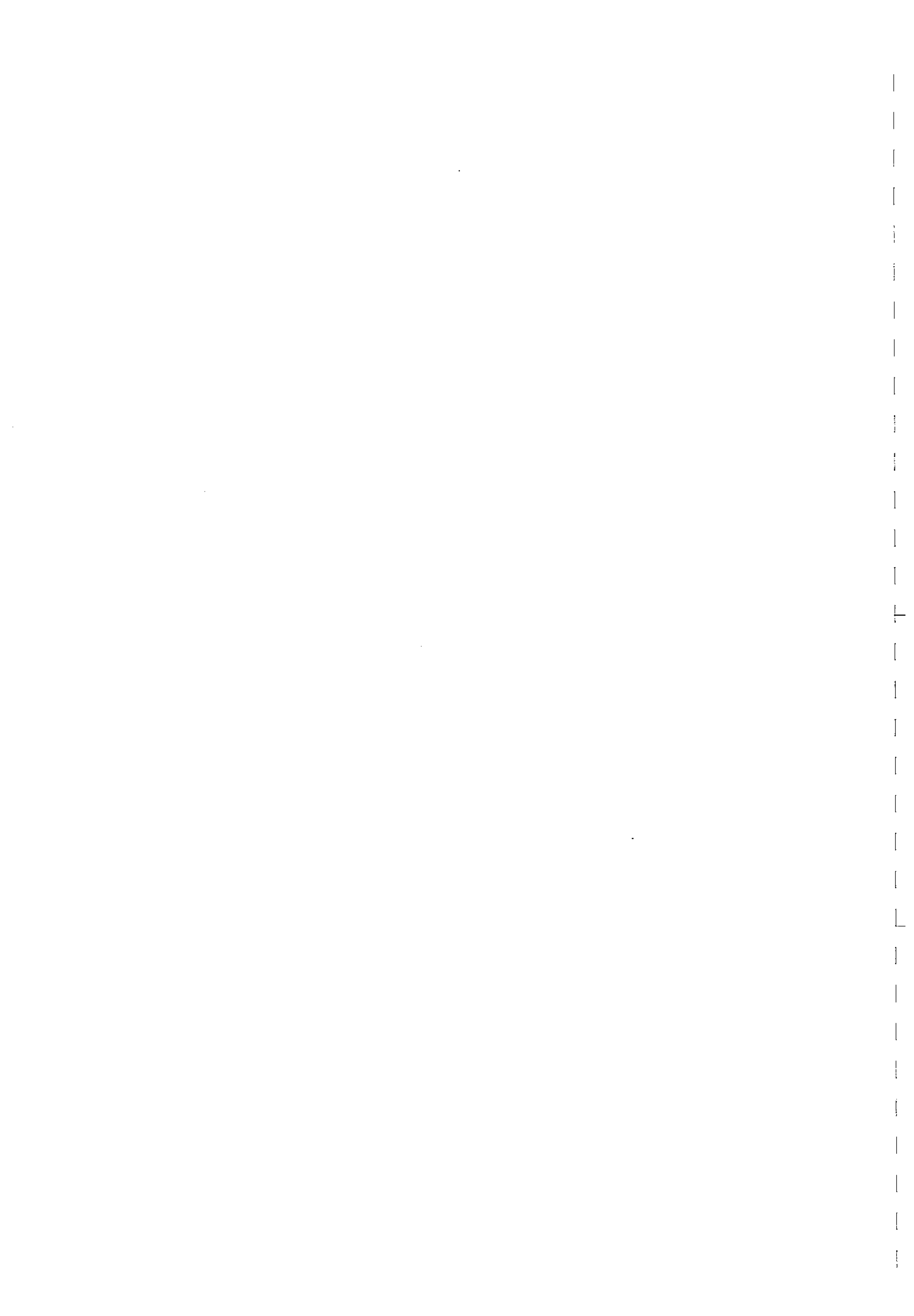
A thermoelectric module consists of numerous pairs of crystalline semiconductor blocks precisely sandwiched between two layers of ceramic substrate (fig. A-1). The blocks are of two varieties: "N-type," which has a surplus of electrons in its crystalline structure, and "P-type," which has a deficit of electrons. The two types are positioned in alternating pairs within the innermost layer of the sandwich.

Figure A-1 A thermoelectric module.



The two types of blocks are wired together in alternating pairs. When electrical current is passed through the blocks, electrons in the N-type blocks and the "holes," or empty electron spaces, in the P-type blocks are excited at one conductor-semiconductor interface, which absorbs a small amount of heat. The electrons and holes flow through the crystalline blocks and return to a low-energy state at the other conductor-semiconductor interface, with the release of the previously absorbed heat. A thermal gradient of up to 70°C can be generated across the blocks in this manner.

The direction of heat pumping is reversed by reversing the polarity of current flow through the thermoelectric module, and the amount of heat pumped is changed by changing the amount of current passed. Both direction and amount of current flow are dictated by a microprocessor in the Alpha™ unit, allowing precise control of thermal cycling in the Alpha unit block.



Appendix B

How a Switching Power Supply Works

Almost all solid-state electronic devices, including the DNA Engine® cycler's thermoelectric module, require direct current (DC) for operation. However, electric utilities supply low-Hertz alternating current (AC), which varies in voltage and frequency from nation to nation. The DNA Engine uses switching transistors, combined with high-frequency, resonant transformers, to convert the incoming AC to DC.

The power supply first chops the AC power into small bursts of energy (over 100,000 per second) with the aid of high-current switching transistors called MOSFETs (metal-oxide semiconductor field-effect transistors). The energy bursts are channeled into a high-frequency transformer. By changing the duration of the bursts that charge the transformer's magnetic core (pulse-width modulation), a specific voltage output can be maintained even when the incoming voltage varies (between 100 and 240 volts in the case of the DNA Engine). Because the incoming power is being chopped so rapidly, the incoming frequency is unimportant; it can even be DC. Spikes and surges in the incoming power no longer pose a problem since they are chopped nearly to oblivion. The addition of resonance to the transformer design gives it extraordinary efficiency. These design innovations has made the DNA Engine's power supply small in size, universal in input, and resistant to noise.

Appendix C

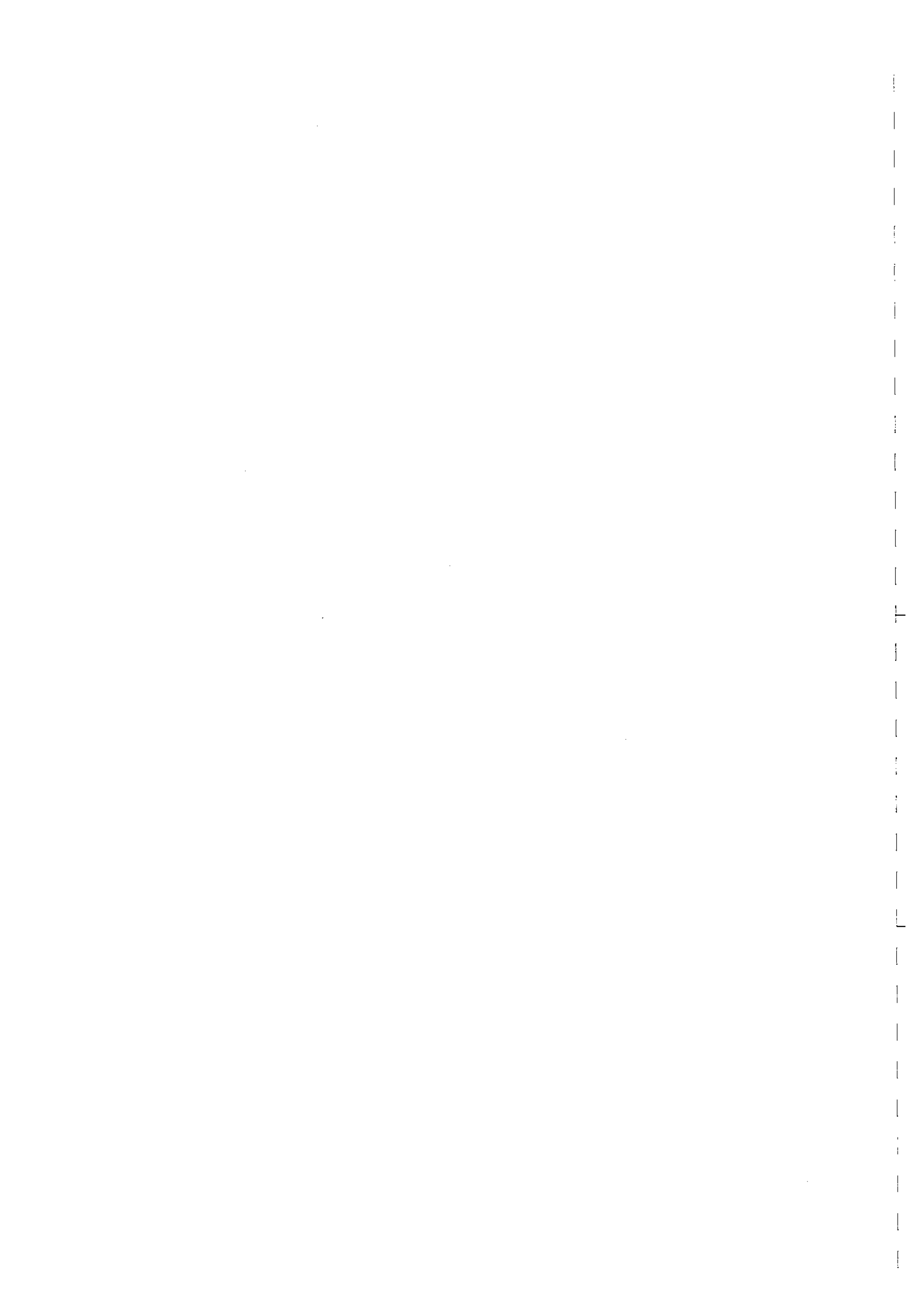
Shipping Instructions for US Residents

Users residing in the United States should follow these instructions for shipping a machine to MJ RESEARCH for factory repair or an upgrade. Users outside of the United States should send machines to their distributor, in accordance with shipping instructions obtained from the distributor.

1. Call MJ RESEARCH to obtain a return materials authorization (RMA) number. Machines returned without an RMA will be refused by the Receiving Department.
2. Thoroughly clean the machine, removing excess oil and radioactive and other biohazardous substances. To protect the health of our employees, MJ RESEARCH will not repair or upgrade any machine that is excessively oily or that emits ionizing radiation upon arrival at our factory. **PLEASE ELIMINATE ALL BIOHAZARDS!**
3. Pack the machine in its original packaging. If this has been misplaced or discarded, call MJ RESEARCH to request shipment of packaging materials. You can also request a loaner machine, which will be provided if available (a rental fee may apply). You can use the loaner's packaging to return the machine needing repair.

   Remove the Alpha unit from the DNA Engine® or DNA Engine Tetrad® base before shipping. All warranties are voided if a machine is shipped with an Alpha unit installed. If the Alpha™ unit also needs to be shipped, pack it in its original packaging materials.
4. Write the RMA number on the outside of the box.
5. Ship the machine (freight prepaid) to the following address. We recommend you purchase insurance from your shipper.

   Ship to: Repair Department
   MJ Research, Inc.
   136 Coolidge Ave.
   Watertown, MA 02172



Appendix D

Warranties

The DNA Engine (PTC-200) and DNA Engine Tetrad (PTC-225) thermal cyclers are warranted against defects in materials and workmanship. For specific Warranty information, contact your local Bio-Rad office. If any defects should occur during the warranty period, Bio-Rad will replace the defective parts without charge. However, the following defects are specifically excluded:

1. Defects caused by improper operation or by improper packaging of returned goods.
2. Repair or modifications done by anyone other than Bio-Rad Laboratories.
3. Use with tubes, plates, or sealing materials not specified by Bio-Rad Laboratories for use with the DNA Engine or DNA Engine Tetrad thermal cyclers.
4. Deliberate or accidental misuse.
5. Damage caused by disaster.
6. Damage due to use of improper solvent or sample.

The warranty does not apply to fuses.

For inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument.

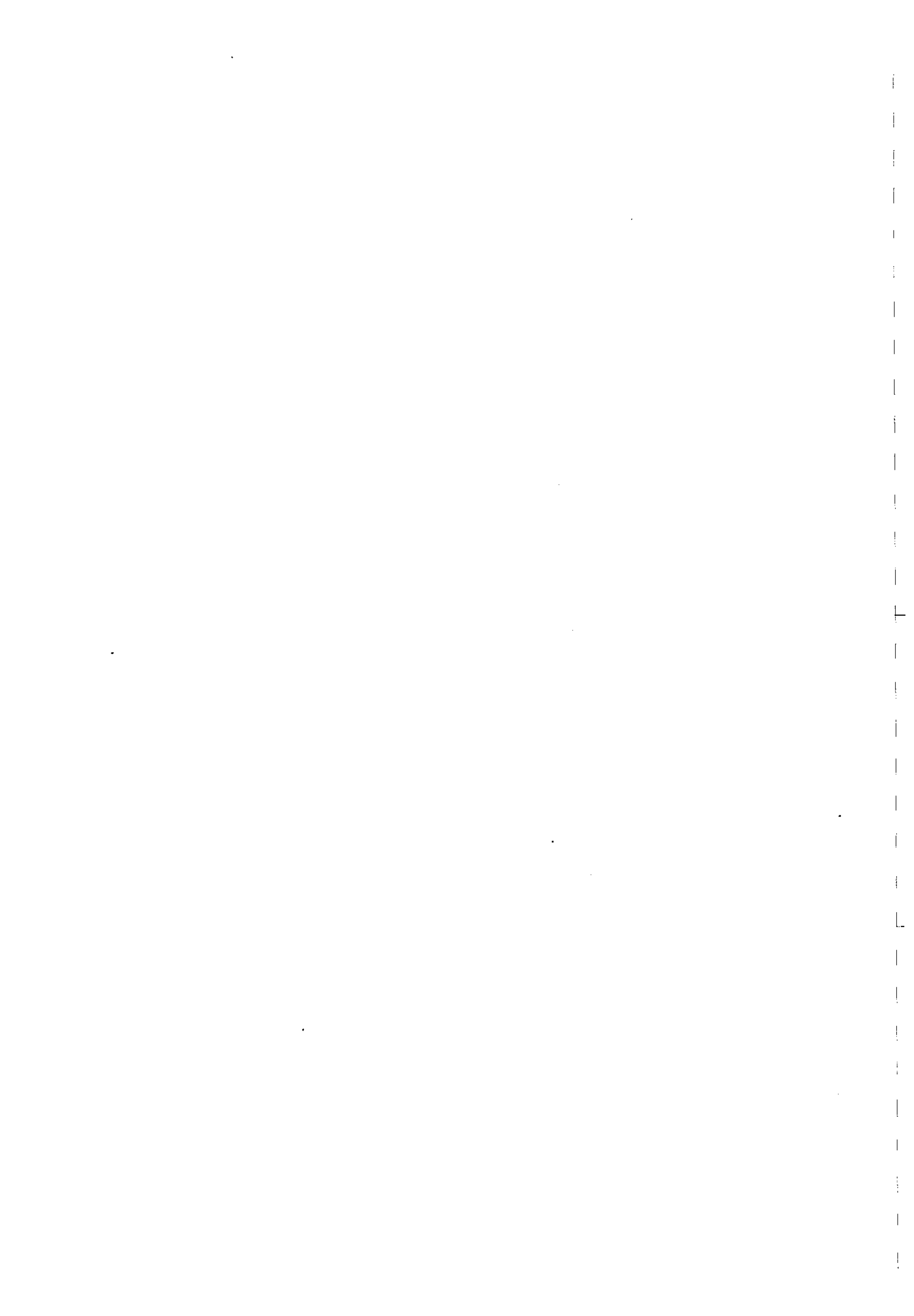
For Technical Service call your local distributor or in the U.S. call 1-888-652-9253, or visit our website at www.mjr.com.

Appendix E

Factory-Installed Protocols

Note: A programmed time of 0:00:00 means "hold at this temperature forever."

Name	Temp. Control Method	Step	Temp. & Time
ICEBUKET	Block	1	4° , 0:00:00
		2	END
LIGATION	Block	1	15° , 0:00:00
		2	END
37° – 1 HR	Block	1	37° , 1:00:00
		2	4° , 0:00:00
		3	END
37° – 6 HR	Block	1	37° , 6:00:00
		2	4° 0:00:00
		3	END
65°	Block	1	65° , 0:00:00
		2	END
DENATURE	Block	1	95° , 0:05:00
		2	END
BOIL	Block	1	100° , 0:05:00
		2	END
CUT&KILL	Block	1	37° , 1:00:00
		2	70° , 0:05:00
		3	END



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Declaration of Conformity

Bio-Rad Laboratories, Inc., 1000 Alfred Nobel Drive, Hercules, California, 94547, U.S.A., declares that the product

PTC-200, The DNA Engine® Thermal Cycler

to which this declaration relates, is in conformity to the following standards or normative documents.

IEC61010-1
EN61326: CLASS A

following the provisions of the 89/336/EEC & 73/23/EEC Directive.

Test Data to verify this conformity are available for inspection at our European Representative Office at Literbuen 10B, 2740 Skovlunde, Denmark.

13 September, 2004
date of issue

Brad Crutchfield
Vice President

11142 rev A.A

Declaration of Conformity

Bio-Rad Laboratories, Inc., 1000 Alfred Nobel Drive, Hercules, California, 94547, U.S.A., declares that the product

PTC-225, The DNA Engine Tetrad® Thermal Cycler

to which this declaration relates, is in conformity to the following standards or normative documents.

IEC61010-1
EN61326: CLASS A

following the provisions of the 89/336/EEC & 73/23/EEC Directive.

Test Data to verify this conformity are available for inspection at our European Representative Office at Literbuen 10B, 2740 Skovlunde, Denmark.

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