PTC-100 Operations Manual

Version 8.1

MJ Research™, Incorporated
Boston • San Francisco • Tahoe • Copenhagen • Seoul

06107-AA

Thermal cyclers can be used for a number of purposes, including the polymerase chain reaction (PCR). PCR is covered by patents owned by Hoffmann-La Roche, Inc., and F. Hoffmann-La Roche, Ltd., who have granted exclusive and nonexclusive licenses for some types of applications. Roche and its licensees provide end-user licenses within their respective fields. These licenses have different terms depending on the particular application of PCR, and different rules may apply in different countries. Anyone who intends to use MJ Research equipment to do PCR is encouraged to contact Hoffmann-La Roche for more information, at one of the addresses below:

In the United States:
Licensing Manager
Roche Molecular Systems, Inc.
1145 Atlantic Avenue
Alameda, CA 94501 USA
(510) 814-2970
Fax: (510) 814-2977

In other nations:
PCR Licensing Manager
F. Hoffmann-La Roche Ltd.
Building 222/350
CH-4002 Basel, Switzerland
41-61-687-3031
Fax: 41-61-687-2113

Contents

Documentation Conventions

Typographic Conventions

The names of keyboard keys are in sans serif type and placed within 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 two lines of text:

Example:

```
RUN        Enter
Program    Program
```

Terminology

A programming option is termed "selected" when the cursor is positioned in front of it. Use the «Select» keys (see fig. 2-3) to move the cursor. In some screens selected items are also displayed in all-capital letters.

1 Introduction

Meet the PTC-100

Thank you for purchasing an MJ Research PTC-100 thermal cycler. Designed by a team of molecular biologists and engineers, the PTC-100 will meet your needs for an easy-to-use, reliable, compact programmable thermal cycler:

- Easy-to-read programming interface for quick and painless programming, editing, file management, and much more
- Choice of block or probe control
- Space-saving design for easy setup and transportation
- Instant Incubate feature for continuous-temperature incubations
- Hot Bonnet heated lid for oil-free cycling
- Customizable factory-installed protocols

How to Use This Manual

This manual contains all the information you need to operate the PTC-100 safely and productively:

- Chapter 2 describes the **physical characteristics and specifications** of the PTC-100.
- Chapters 3–5 describe **installing and operating** the PTC-100.
- Chapters 6 and 7 describe **programming** the PTC-100.
- Chapter 8 describes proper **maintenance** of the PTC-100.
- Chapter 9 offers advice on **troubleshooting** the PTC-100.

Note: Information on installing, operating, and programming the 16MS Slide Block cycler may be found in appendix A.

Important Safety Information

Safe operation of the PTC-100 begins with a complete understanding of how the machine works. Please review this entire manual before attempting to operate the PTC-100. Do not allow anyone who has not reviewed this manual to operate the machine.

The PTC-100 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 in appendix B, and exercise all precautions outlined in them.

2

Layout and Specifications

PTC-100 with Cold Lid

Models Available

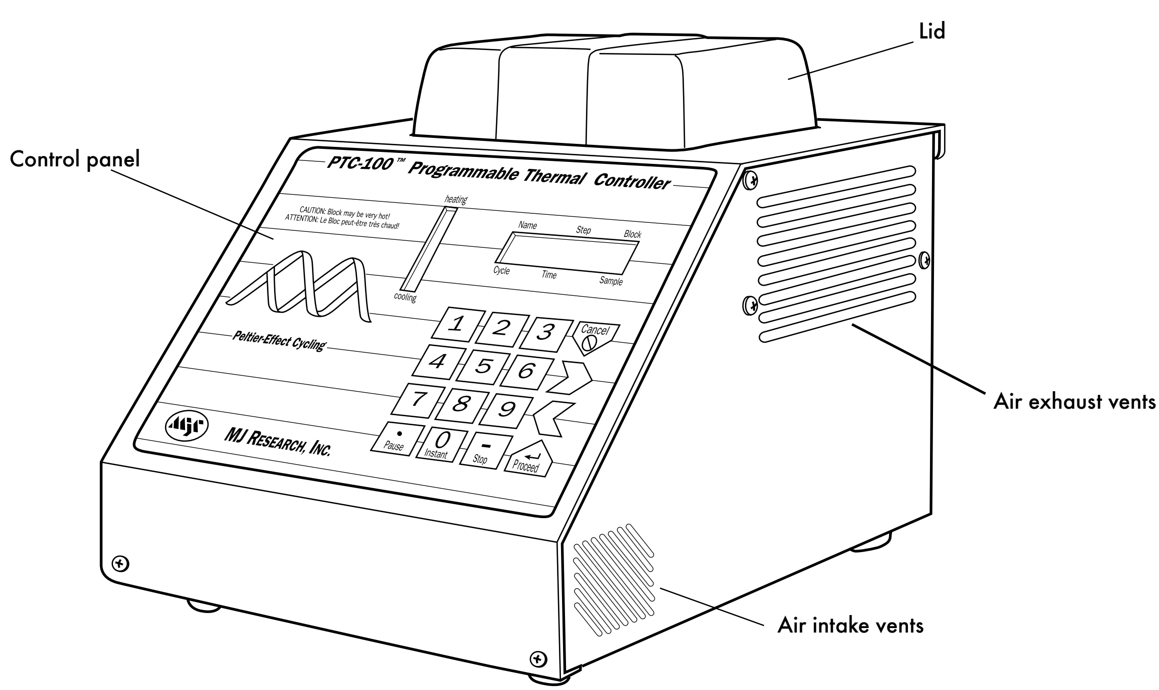
60-well block: holds 60 x 0.5ml tubes

96-well block: holds 96 x 0.2ml tubes or one 96-well microplate

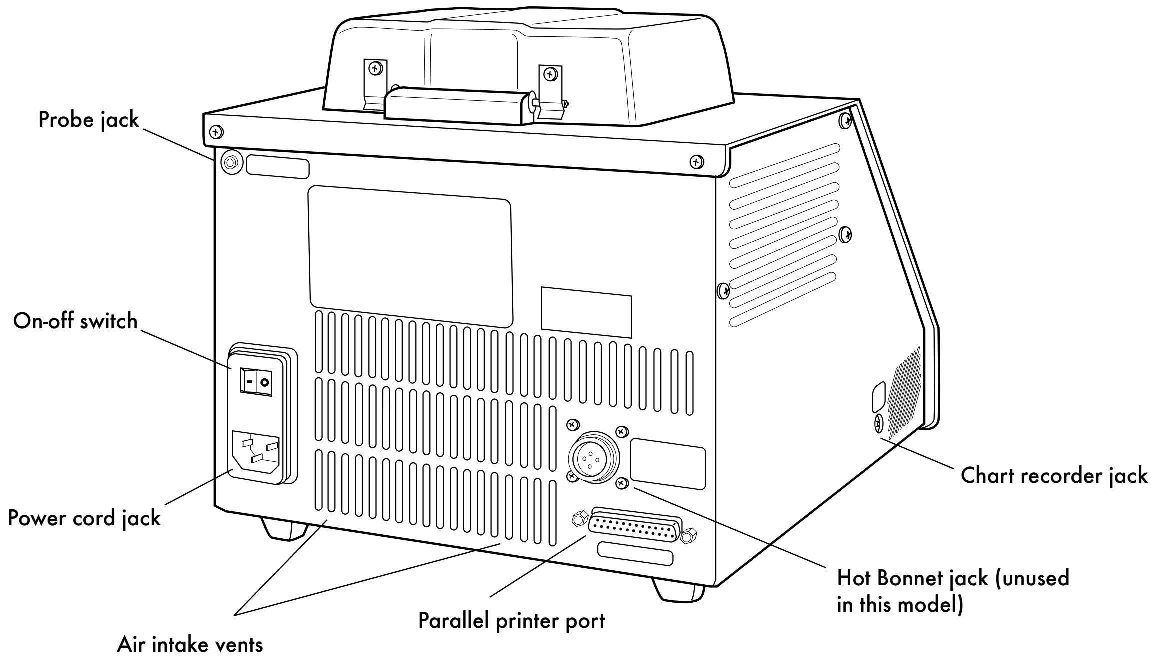
96AgV gold/silver block: holds 96 x 0.2ml tubes or one 96-well microplate; block is gold-plated sterling silver for faster ramping speed

16MS Slide Block: holds sixteen 25 x 75mm slides and 24 x 0.2ml tubes (see p. 2-6)

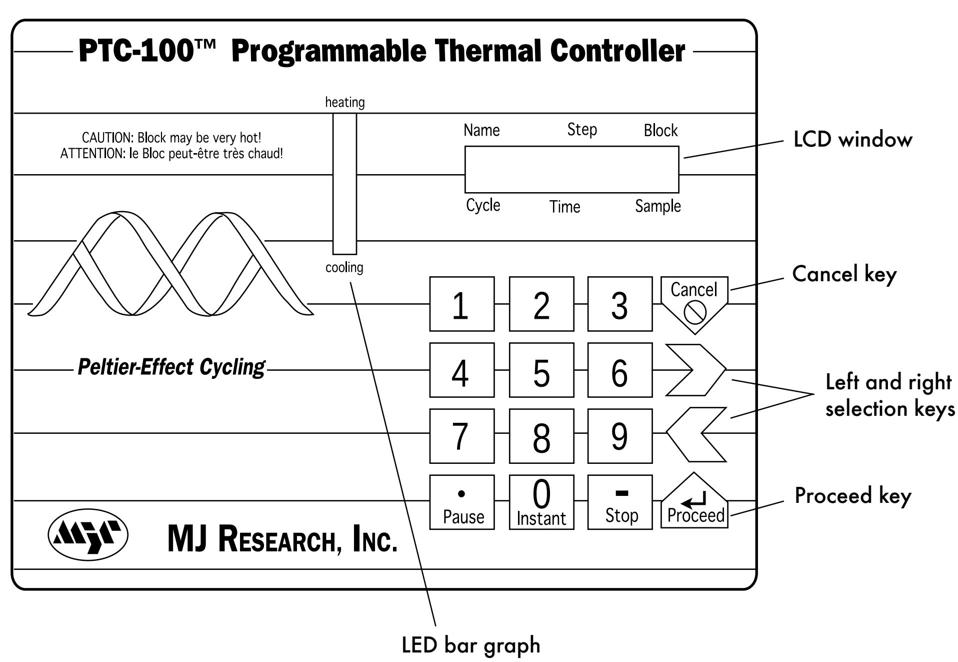
Front View (Fig. 2-1)



Back View (Fig. 2-2)



Control Panel (Fig. 2-3)



PTC-100 with Hot Bonnet

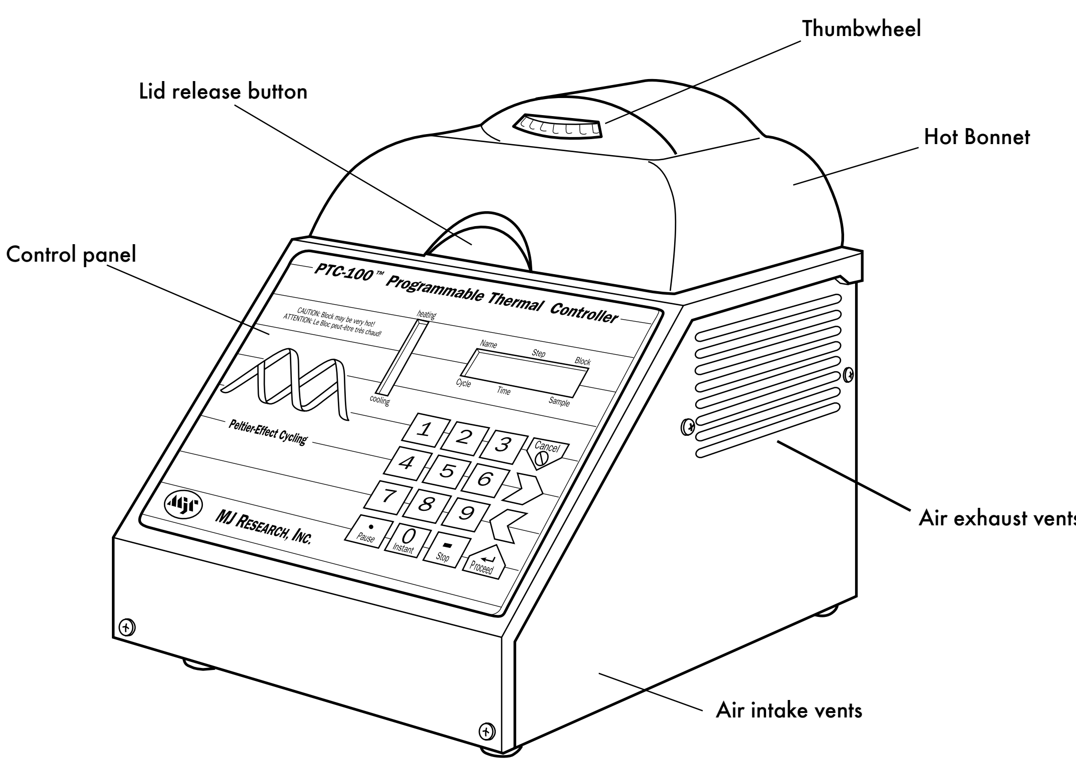
Models Available

60-well block: holds 60 x 0.5ml or 0.6ml microcentrifuge tubes

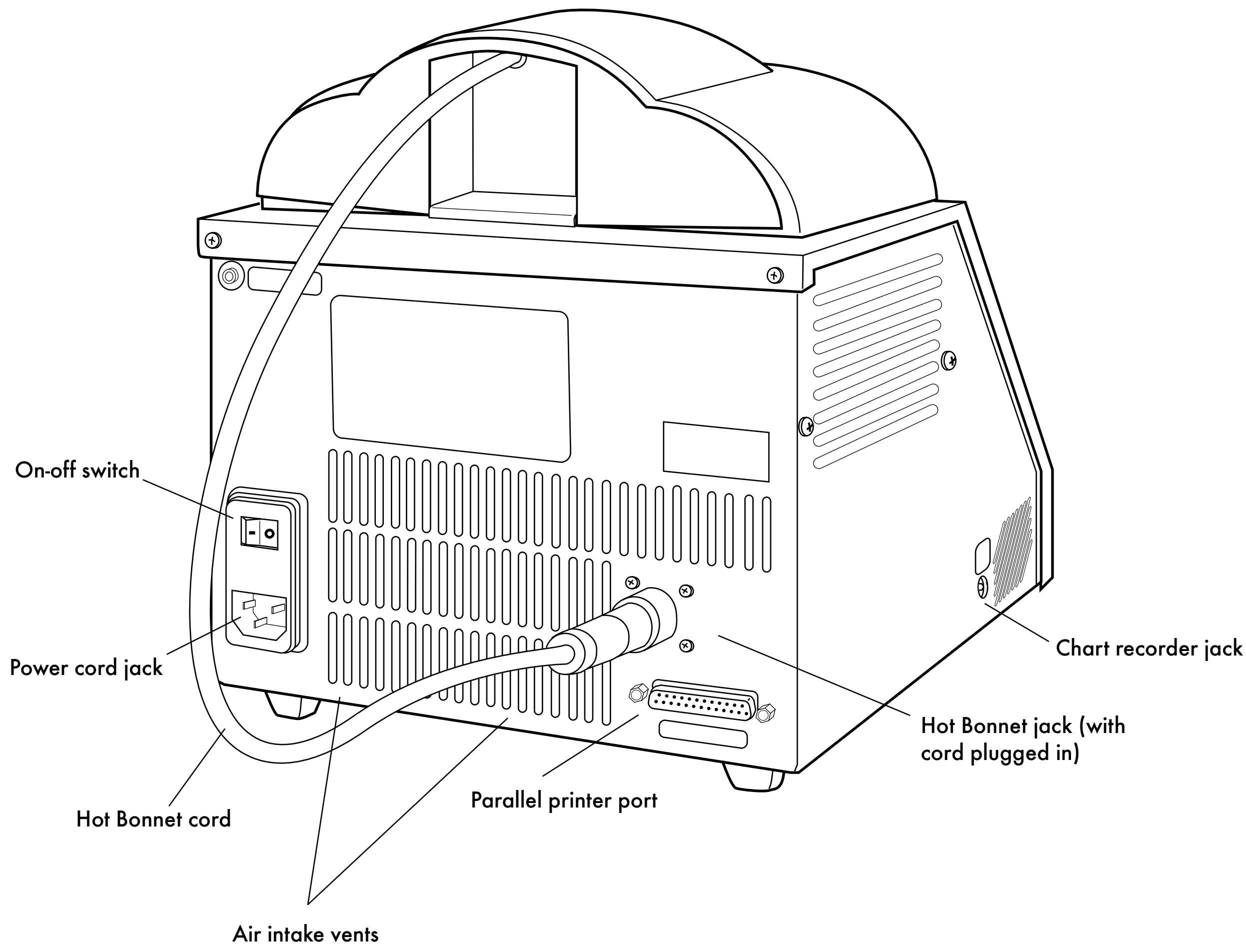
96-well block: holds 96 x 0.2ml tubes or one 96-well microplate

96AgV gold/silver block: holds one 96-well microplate or 96 x 0.2ml tubes; block is gold-plated sterling silver for faster ramping speed

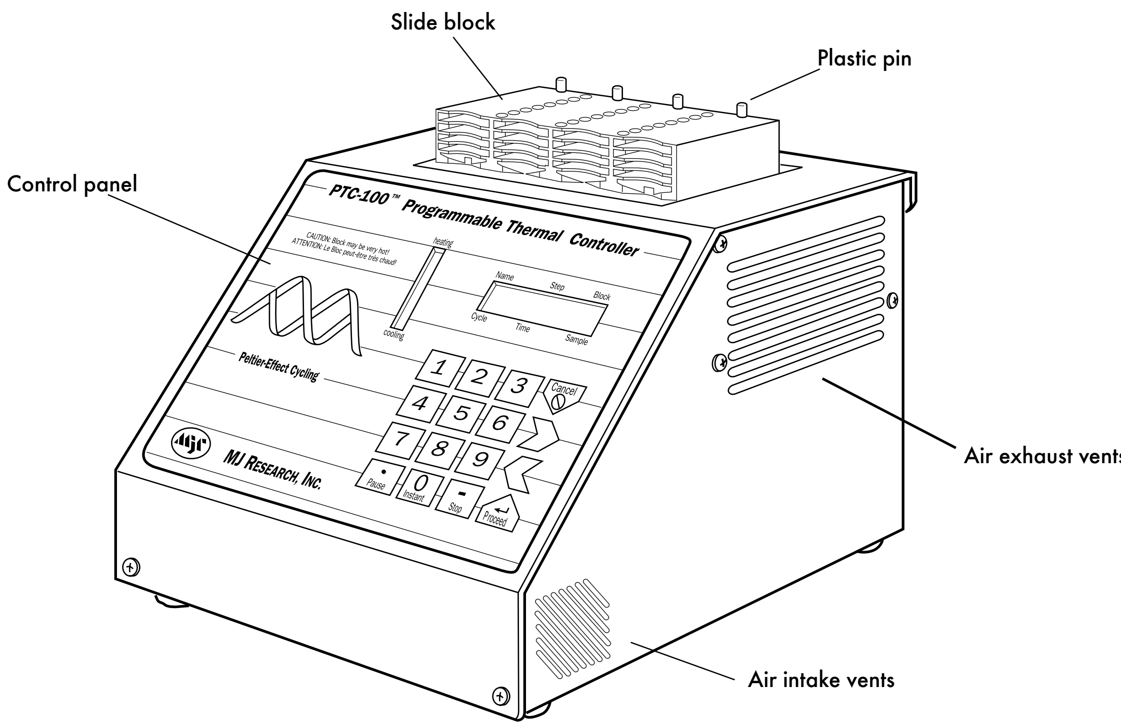
Front View (Fig. 2-4)



Back View (Fig. 2-5)



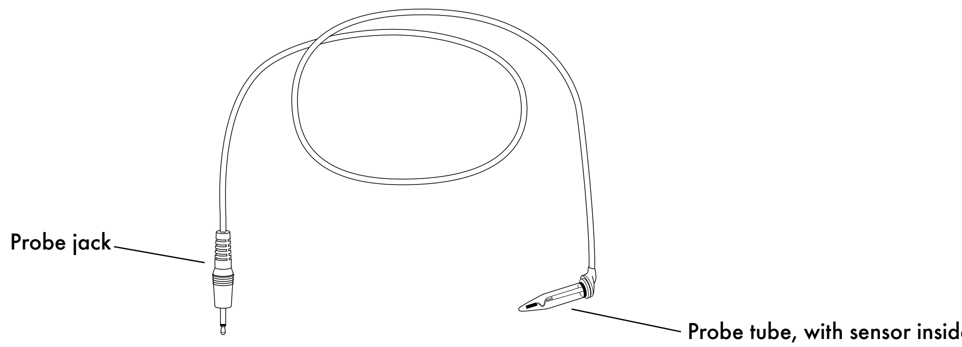
PTC-100 with 16MS Slide Block
(Fig. 2-6)



Accessories

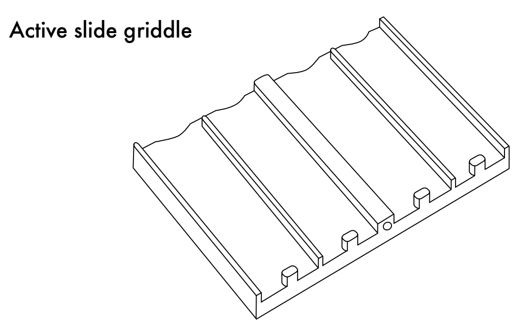
Temperature Probe (Fig. 2-7)

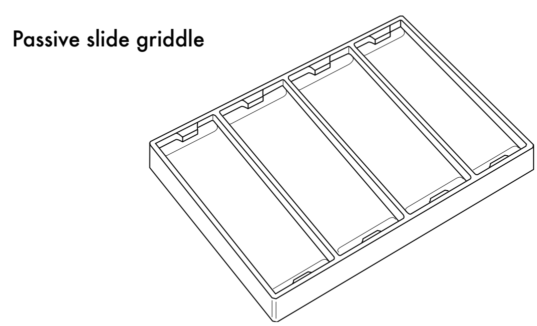
Allows machine to control reactions according to a representative sample's temperature. Available for the 60-well model of the PTC-100, with or without the Hot Bonnet.



Slide Griddle Adapter (Fig. 2-8)

Allows machine to thermally cycle up to four 25 x 75mm glass slides. Two models are available: the actively controlled adapter and the passive adapter. Available for 60-well and 96-well models of the PTC-100, with or without the Hot Bonnet.





Specifications

Thermal range:	0–100°C
Accuracy:	±0.5°C of programmed target at 60°C
Thermal homogeneity:	±0.4°C well to well within 30 seconds of arrival at 60°C
	16MS slide block: ±0.4°C within 30 seconds of arrival at 90°C
Ramping rate:	**60-well block:** up to 1°C/sec
	96-well: up to 1.2°C/sec
	96AgV gold/silver block: up to 2.5°C/sec
	16MS Slide Block: up to 0.5°C/sec
Sample capacity:	**60-well block:** Sixty x 0.5ml tubes
	96-well block, 96AgV gold/silver block: 96 x 0.2ml tubes or one 96-well plate
	16MS Slide Block: Sixteen 25 x 75mm slides and 24 x 0.2ml tubes
Line voltage:	100–240VAC rms (no adjustment needed among voltages within these ranges)
Frequency:	50/60Hz single phase
Power:	350W maximum (momentary 4.5 A)
Fuses:	Two 4A, 5 x 20mm (4 ampere)
Displays:	One 2 x 16 LCD alphanumeric display
Ports:	One 25-pin 8-bit parallel interface printer port
Memory:	360 typical programs, in nonvolatile memory; programs may contain up to 100 steps; maximum of 1600 steps among all programs
Weight:	7.0kg
Size:	PTC-100 with cold lid, 24cm x 28cm x 23cm; PTC-100 with Hot Bonnet, 23cm x 28cm x 26cm

3 Installation

Packing List

After unpacking the PTC-100, make sure you have received the following:

- One PTC-100, with Hot Bonnet if ordered
- One power cord
- Two fuses
- One sponge rubber pad (with 96-well block, for cold lid machines only)
- *The PTC-100 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 PTC-100 to obtain a replacement. Please save the original packing materials in case you need to return the PTC-100 for service. See appendix E for shipping instructions.

Setting Up the PTC-100

Insert the power cord plug into its three-pronged jack at the back of the machine (see fig. 2-2), then plug the cord into an electrical outlet (see "Power Supply Requirements," below).

For the PTC-100 with Hot Bonnet, screw the Hot Bonnet power cord into its jack at the back of the machine (see fig. 2-5; finger-tightness is adequate).

Situate the machine according to the instructions below.

Environmental Requirements

Ensure that the area where the PTC-100 is installed meets the following conditions, for reasons of safety and performance:

- Indoor use, nonexplosive environment
- Normal air pressure (altitude below 2000m)
- Ambient temperature 4–32°C
- Humidity10–90%
- Protection from excessive heat (e.g., radiators) and accidental spills

Power Supply Requirements

The PTC-100 requires 100–240VAC, 50–60Hz, 850W, and a grounded outlet. The machine can use current in the specified range without adjustment, so there is no voltage-setting switch.

Air Supply Requirements

Air is taken in from vents at the rear and sides and on the bottom of the machine and exhausted from vents at both sides of the machine. If the machine does not get enough air or gets air that is too warm, it can overheat. Overheating can cause the machine to display a warning message, “H[eat] S[ink] Overheating, Check Air Flow,” or even to shut itself off.

To prevent overheating, position the machine at least 10cm from other thermal cyclers or walls. If the ambient air temperature exceeds 32°C, use fans or adjust the air conditioning to cool the air that the machine receives. Keep the air intake vents clean and free of obstructions such as loose sheets of paper.

4 Operation

Turning the PTC-100 On

Move the power switch at the right rear of the machine to “1” (the “On” position). In most cases, a self-test of the heat pump will begin running (see note below). Its progress is tracked in a screen in the LCD window:

```
SELF TEST
*
```

This screen disappears within 10 seconds. If a problem is detected, an error message is displayed.

Note: If either the heat sink or the block is not between 17 and 30°C, the machine will skip the self-test.

Following the self-test, the Run-Enter Menu is displayed:

```
_RUN      Enter
Program  Program
```

The PTC-100 is now ready to execute programs.

Using the Control Panel

The control panel (see fig. 2-3) 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.
- **Proceed:** Accepts a selected menu or screen option.
- **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 PTC-100 as a simple incubator.

Status Indicator Lights

- **Power light:** Glows red when the PTC-100 is powered up.
- **LED bar graph:** Glows red along its top half when the machine is heating; glows green along its bottom half when the machine is cooling.

Opening and Closing the Lid

Opening the 60-block PTC-100 with cold lid: Lift the lid (there is no latch) (fig. 4-1A, p. 4-4).

Opening all other PTC-100s with cold lids: Gently push on the metal latch at the front of the lid (fig. 4-1B) until it disengages, then lift the lid.

Opening the PTC-100 with Hot Bonnet: Push on the blue button at the lid's center front and lift the lid (fig. 4-1C).

Closing all PTC-100 models: Push the lid down to cover the block. Make sure that lid pressure is correctly adjusted on Hot Bonnet models.

Selecting the Correct Sample Vessel

MJ Research offers a full range of tubes and microplates, manufactured to the specifications of each type of block to ensure a precise fit. See chapter appendix 4-A for a complete list. 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.

0.5ml Tubes

Make sure thick-walled 0.5ml 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 0.5ml 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 0.5ml tubes designed for precise block fit.

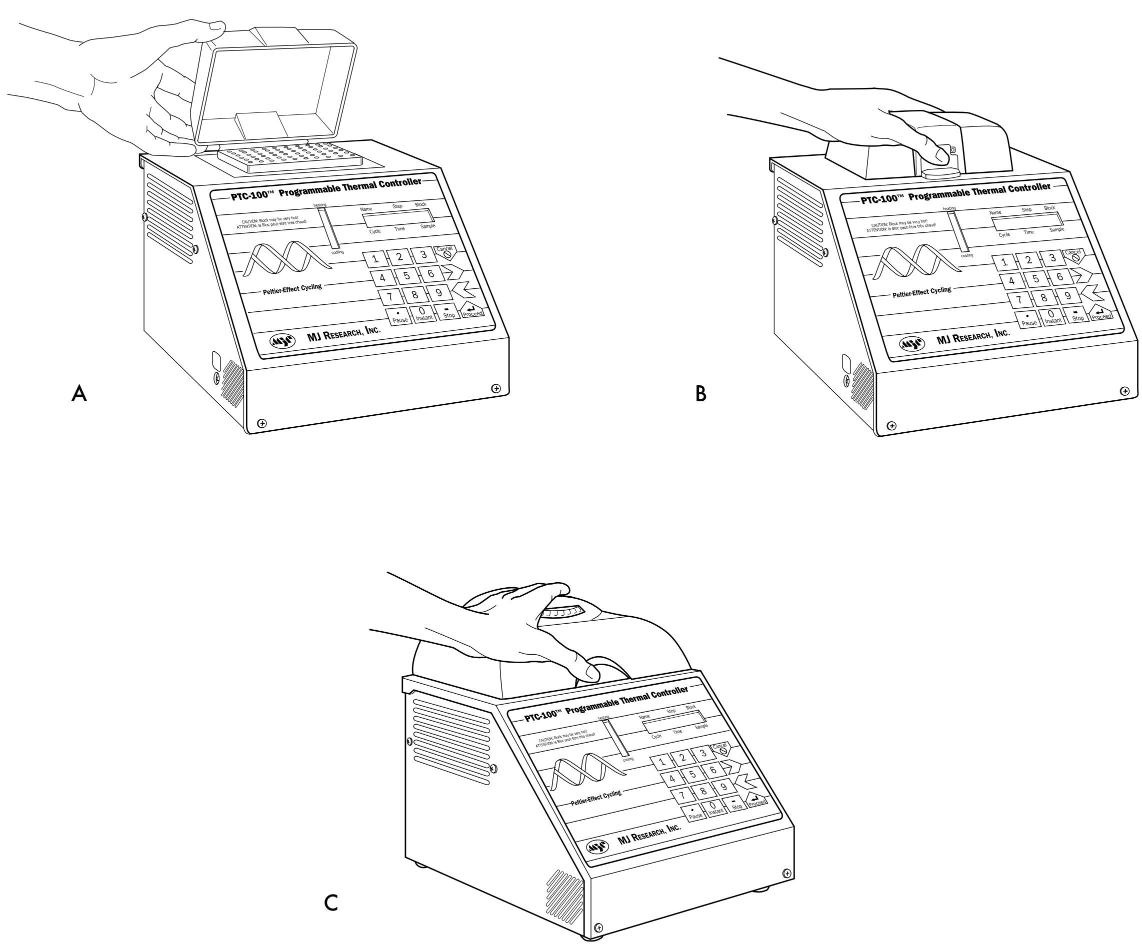
0.2ml Tubes

All types of thin-walled 0.2ml 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 polypropylene or polycarbonate microplates may be used in 96-well blocks 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," p. 4-6, for a description of Microseal 'A'.)

Figure 4-1 A, Opening the 60-well block PTC-100 with cold lid. B, Opening all other PTC-100s with cold lids. C, Opening the PTC-100 with Hot Bonnet.



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. Thicker 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.

Many 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 4-2 (p. 4-6), 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, and this sealing method must be used when cycling samples in PTC-100s that do not have the Hot Bonnet. However, an adequate degree of protection can be achieved by sealing with Microseal film, mats, or caps, then cycling the samples using the Hot Bonnet heated lid to prevent condensation/refluxing. (See appendix A for information on sealing slides for use in the 16MS Slide Block.)

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. **Use the same amount of oil or wax in all sample vessels to ensure a uniform thermal profile.**

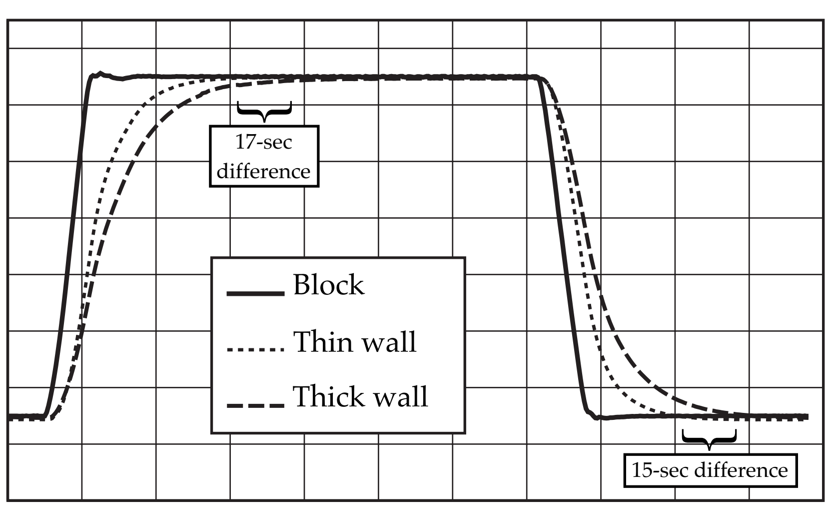
Some paraffin waxes solidify at room temperature. The wax can then be pierced with a micropipette and the samples drawn off. Silicone oil and mineral oil can be poured off or aspirated from tubes if the samples are first frozen (–15 to –20°C). The samples are usually pure enough for analysis without 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 step 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 known adverse effects on fluorescent gel analysis of reaction products.

Sealing with the Hot Bonnet and Caps or Film

The Hot Bonnet heated lid (available for all PTC-100 blocks except the 96U block and the 16MS Slide Block) maintains the air in the upper part of sample vessels at a higher temperature than the reaction mixture. This prevents condensation, refluxing, and changes in reactant concentrations. 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.

Figure 4-2 Ramping rate enhancement with thin-walled tubes. To compare ramping rates of sample temperature, 50µl samples of water were cycled in 0.5ml thin- and thick-walled tubes in a PTC-200 thermal cycler with a 60-well block. Thermal profile: 94°C, 120 sec; 62°C, 120 sec. Data are shown for the third cycle. The cycler was run under block control, and the Hot Bonnet was heated to 105°C and tightened to a half-turn past the touch point. The samples in thin-walled tubes reached the target temperature more quickly.



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 or microplate wells above the liquid level but below the top of the 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 a given type of tube or microplate, sealed in a given way. 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 tubes or a microplate, which would prevent the lid from exerting uniform pressure on the loaded tubes or microplate.
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 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 according to the manufacturer's directions.

4. Close the Hot Bonnet. Turn the thumbwheel clockwise to lower the Hot Bonnet's inner lid onto the loaded tubes/microplate. 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 tubes/microplate.
5. Open the Hot Bonnet. Turn the thumbwheel clockwise an extra half to three-quarters of a turn to set an appropriate lid pressure.

   Note: 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 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.

   ✔ **Tip:** 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. You will hear a click when the lid latch engages. If the latch does not engage, the lid is not closed. Firm pressure may be required to engage the latch.

Loading Sample Vessels

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. If using the Hot Bonnet, also load at least one empty tube in each corner of the block, to ensure that the Hot Bonnet exerts even pressure on the tubes (see "Adjusting the Hot Bonnet's Lid Pressure," p. 4-7).

To obtain 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:

- Check that the block is clean before loading samples (see chapter 8 for cleaning instructions).

- Firmly press the tubes or microplate into the block wells.

Note: See appendix A for information on loading slides into the 16MS Slide Block.

Using the Sponge Rubber Pad with the 96-well Block

In PTC-100 models with the 96-well block and the cold lid, a sponge rubber pad may be used to help seat tubes or a plate into the block. Attach the magnetic backing of the pad to the inner surface of the lid. When the lid is closed, the pad will press loaded tubes or plate down into the wells.

! **Caution:** Never use the sponge rubber pad with the Hot Bonnet heated lid. The pad will block transmission of heat to the tops of loaded vessels, causing reaction failures. The heated lid is able to firmly seat vessels into the block, without the assistance of the pad.

Using Oil to Improve Thermal Contact

A small amount of oil may be placed within block wells to improve heat transfer from the block to loaded vessels and to help prevent the escape of radioactive ^{35}S nucleotides when using polypropylene tubes and polypropylene or polycarbonate microplates (see chapter appendix 4-B).

Using oil requires care, attention, and cooperation on the part of everyone who uses the machine, in order to avoid the problems associated with its use:

- It can be messy.
- If used in excess of recommended amounts, it can seep into the machine and damage internal components.
- It must be used consistently. If a protocol is designed to be run with oil in the block, oil must **always** be used for the protocol in order to ensure replicable results.

The first time you put oil into the block, put two drops into each well that will be holding a sample vessel. Thereafter, more oil must generally be added every two to three times the machine is used.

Each time you run a protocol requiring oil, make sure sufficient oil is present in the wells:

- **When using tubes:** Insert a tube, remove it, and visually inspect for the presence of oil on the outside of the tube. If none is visible, add a drop of oil to the well. Repeat for each tube loaded.

- **When using a plate:** Follow the same procedure as for tubes. If no oil is visible on the outside of the plate, add a drop of oil to each well in the block.

Excess oil in any well will overflow onto the top surface of the block when tubes or a plate is loaded. If this occurs, remove the excess oil with a tissue or pipettor. Do not allow oil to accumulate on the block's top surface.

Note: The Hot Bonnet makes it unnecessary to use oil in the block, because it presses tubes into the block wells, which improves thermal contact.

Using the Slide Griddle

The Slide Griddle heats up to four glass slides for *in situ* protocols. Two models are available, actively controlled and passively controlled. The actively controlled model uses a temperature probe to allow the machine to precisely control the Slide Griddle's temperature. The actively controlled Slide Griddle can only be used with PTC-100s that have the 60 block, since this model of the machine has a temperature probe jack. The passively controlled model does not use a temperature probe, so it may be used with PTC-100s that have the 96-well block or the 60 block.

See the *Slide Griddle Operations Manual* and *Passive Slide Griddles for MJ Research Thermal Cyclers* for instructions on operating both types of Slide Griddle.

Using the Probe

An in-sample temperature probe is available for PTC-100s with the 60-well block (both cold lid and Hot Bonnet models). The probe consists of a semiconductor sensor mounted in a 0.5ml plastic tube (see fig. 2-7). A wire runs from the sensor to the probe's plug, which is inserted into a jack at the back of the machine. 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 4 and 96°C.

When a probe-control protocol is run, the PTC-100 controls the block's temperature to keep the probe at the programmed temperature, using feedback information from the sensor. Protocols must be tailored to fit this control mechanism (see chapter 6).

Customizing the Probe Vessel

For the most precise control of sample temperatures, install the probe sensor 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. Uncap the probe tube. The sensor will come out when the lid is removed.
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 sensor in the new tube, and snap the lid closed. Make sure that the lid from the original probe tube fits the new tube tightly and that the tube is long enough to accommodate the sensor wire. The sensor should rest on the bottom of the tube.

! Caution: The sensor and the wires connecting it to the probe are fragile. Handle them with great care. In particular, do not bend the wires connecting the sensor to the main probe wire.

Adding the Oil

Viscous oils (not water!) 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.

Light and heavy mineral oil and silicone oil may be used (Table 4-1). MJ Research recommends using heavy mineral oil because the formula for determining the correct volume of oil to use is easy to remember, and it is widely available and inexpensive. But whichever type of oil you use, **be careful** to add the correct amount of oil.

Table 4-1 Calculating the correct amount of oil

Type of oil	Sigma number	Amount to use
Heavy mineral oil	400-5	1 x volume of buffer in individual sample tube + 1 x volume of oil overlay
Light mineral oil	M5904	1.4 x volume of buffer in individual sample tube + 1 x volume of oil overlay
Silicone oil	DMPS-5X	1.7 x volume of buffer in individual sample tube + 1 x volume of oil overlay
Silicone oil	DMPS-V	2.7 x volume of buffer in individual sample tube + 1 x volume of oil overlay

Loading and Connecting the Probe

Seat the probe tube in the center of the block. If oil is used to thermally couple samples to the block, it must also be used on the probe tube.

Plug the probe into the jack at the back of the instrument (see fig. 2-2). Close the lid, making sure that the probe wire exits the lid through the small hole provided for it.

When the probe is connected to the machine, the probe calibration screen will be displayed every time *Run Program* is selected from the Run-Enter menu (see “Calibrating the Probe,” below).

Running a Protocol under Probe Control

To run a protocol under probe control, load and connect the probe as described above. Select a protocol that has been designed for probe control and press «Proceed». The following screen will be displayed:

```
In-sample Probe
Control? _NO Yes
```

Select *Yes* and press «Proceed». The protocol will begin running under probe control.

Calibrating the Probe

Calibrate the probe for the following conditions:

- the first time it is used and once a month thereafter
- whenever there is a discrepancy of 1°C or more between block temperature and probe temperature at a steady-state, near-ambient temperature
- whenever it takes longer than 20 seconds for the block to equilibrate at a new temperature, when running a probe-control program

For greatest accuracy, recalibrate the probe whenever the sample volume changes.

To calibrate the probe, remove all samples from the block. Fill the probe with enough oil to completely cover the sensor. Firmly seat the probe in the center of the block, and plug it into the machine. Close the machine’s lid, select a program, and press «Proceed». The following screen will be displayed:

```
Calibrate Probe?
N/Y Takes 10 Min
```

Select *Y* and press «Proceed». The following screen will be displayed:

```
REMOVE YOUR
SAMPLES
```

Remove all samples from the block, then press «Proceed». The following screen will be displayed:

```
Check Probe Prep
Insert in Block_
```

If the probe is filled with the correct amount of oil and inserted into the block, press «Proceed». The calibration process will begin. The machine will cool the probe sample to 4°C for 3 minutes, then heat it to 96°C for 3 minutes. A screen will be displayed during the calibration, showing the block temperature in the upper right-hand corner and the probe temperature in the lower right-hand corner:

```
CALIBRATING 23.2
            24.5
```

When the calibration is completed, you may load samples and run a protocol.

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

Key

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

MJ Research Thermal Cycler Blocks			Reaction Vessels		Sealing Options for Oil-Free Cycling					
96V (0.2ml)	60 (0.5ml)	16 slide	Description	MJ Research Catalog #	Microseal 'A' film MSA-5001	Microseal 'M' mat* MSM-1001	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	TBI-0601						
		●	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 positioned symmetrically in the block.

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 exhibits some vapor loss through the plate walls.

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, 0.2ml tubes, and 0.5ml 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.

0.2ml 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 0.2ml tubes of several different brands during thermal cycling. No data are yet available on radioactive contamination with polypropylene microplates.

0.5ml 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.

5 Running Protocols

Running a Protocol

Either a custom-designed protocol or one of the factory-installed protocols may be run. See appendix C for descriptions of the factory-installed protocols, which may be edited to fit your needs.

Select *Run Program* from the Run-Enter Menu, then press «Proceed».

Press one of the «Select» keys until the name of the protocol you wish to run is displayed, then press «Proceed». The protocol will begin running.

```
Run
QUIKSTEP?
```

Running a Protocol on the PTC-100 with Hot Bonnet

Select a protocol to run and press «Proceed». The following screen will be displayed:

```
_ENABLE Disable
  Heated Lid
```

To enable the Hot Bonnet, first make sure it is plugged into its jack (see fig. 2-5). Select *Enable* and press «Proceed». The protocol will begin to run, and the Hot Bonnet will be turned on.

Note: If the Hot Bonnet is not plugged into the machine and you enable it and run a protocol, the machine will begin beeping.

If you do not wish to use the Hot Bonnet, select *Disable* and press «Proceed». The protocol will begin to run, without the Hot Bonnet.

Running a Protocol under Probe Control

To run a protocol under probe control, load and connect the probe (see p. 4-12). Select a protocol that has been designed for probe control. Do not use probe control to run a protocol designed for block control. Press «Proceed». The following screen will be displayed:

```
In-sample Probe
Control? _NO Yes
```

Select *Yes* and press «Proceed». The protocol will begin running under probe control.

If probe control is not desired, select *No* and press «Proceed». The protocol will begin running under block control.

Reading the Runtime Screen

During a protocol run, a runtime screen will be displayed:

```
CUSTOM1   3   65.4
2             64.0
```

This screen lists the program name (CUSTOM1 in the example above), the protocol step that is running (3), the block temperature (65.4°C), the cycle number (2), and the sample temperature if the probe is installed (64.0°C).

When the target temperature for a step is reached, a timer begins running in the middle of the second line:

```
CUSTOM1   3   92.0
2      00:10 92.1
```

The timer shows the length of time the samples have been held at the displayed temperature. When another step begins, the timer disappears until the new step's target temperature is reached. At that point the timer begins running for the step.

Reading the LED Bar Graph

As the protocol runs, the LED bar graph indicates how much power is going to the block's heat pump. The upper half of the bar lights up red when the block is heating, and the lower half lights up green when the block is cooling. The percentage of the column that lights up during either heating or cooling provides an instantaneous reflection of how much energy the heat pumps are using to either heat or cool the block.

Reading the Protocol Completion Screen

When the protocol ends, a beeper sounds and a message is displayed:

```
CUSTOM1  3  92.0
COMPLETE
```

The temperature of the block at the completion of the program is displayed in the upper right-hand corner of the screen. The number will change as the block cools to ambient temperature.

Press «Proceed» to remove this screen. The Run-Enter Menu will be displayed, and another protocol may be run.

Manually Stepping Through a Protocol

A running protocol can be manually advanced through its steps. As soon as a step has reached its target temperature (i.e., when the timer begins running for the step), press «Proceed» to progress the protocol to its next programmed step. The next protocol step will immediately be displayed.

Note: Pressing «Proceed» while a step is ramping will have no effect. A step must reach its target temperature before the protocol can be manually advanced to the next step.

Protocols can be programmed to require manual stepping (see p. 6-10).

Pausing a Running Protocol

Press «Pause» to temporarily stop a running protocol. If «Pause» is pressed during temperature ramping, the protocol will pause as soon as the target temperature is reached, and samples will be held at the displayed temperature. If «Pause» is pressed after a step has reached its target temperature, the protocol will immediately begin holding samples at the current incubation temperature. The timer will stop running, and the word "Pause" will be displayed:

```
CUSTOM1  3  92.0
2     PAUSE
```

To resume the protocol, press «Pause» again. The timer will begin running again, and the protocol will complete the step that was paused and continue on to the next step.

Stopping a Running Protocol

Press «Stop» or «Cancel» to stop a running protocol. A beeper will sound, and the program will stop running. The program cancellation screen will be displayed:

```
CUSTOM1  2  75.2
CANCELED BY USER
```

The current block temperature is displayed in the upper right-hand corner of this screen. The reading will change as the block returns to ambient temperature. Press «Proceed» to remove this screen. The Run-Enter Menu will be displayed.

Note: Turning off the machine will not cancel a running protocol. Instead, the PTC-100 will assume that a power outage has occurred and will resume the protocol run when the machine is turned on again (see below).

Resuming a Protocol after a Power Outage

If a power failure occurs when a protocol is running, the PTC-100 will hold the protocol in memory for at least 24 hours and sometimes up to 10 days. (The exact duration depends on environmental conditions.)

When power is restored, the protocol will begin running again at the point at which it was stopped. When the protocol ends, the protocol completion screen will be displayed, with a special notice on its last line:

```
CUSTOM1 Complete
PRESS PROCEED
```

Press «Proceed» as instructed. A screen about the power outage will be displayed. The screen will identify the step and the cycle that were running when the power failure occurred:

```
AC POWER FAILED
Cyc 3     Step 2
```

Press «Proceed» to remove this screen. The Run-Enter Menu will be displayed again.

Running an Instant Incubation

The PTC-100 may be converted to a constant-temperature incubator by pressing «Instant» (the zero key) while the Run-Enter Menu is displayed. A screen allowing entry of the incubation temperature will be displayed:

```
TEMP:_
```

Type any incubation temperature from 0.0°C to 100.0˚C, then press «Proceed». The PTC-100 will incubate the sample at the specified temperature.

When the block reaches the incubation temperature, a timer will begin running in the lower right-hand corner of the screen. To stop and start the timer, press «Pause». To stop an instant incubation, press «Cancel» or «Stop» (see "Stopping a Running Protocol").

Printing a Log of a Running Protocol

If the PTC-100 is connected to a printer, it will print a log for a running protocol that includes

- The machine's serial number and software version
- The hours of operation and calibration data
- The protocol's name and a list of its steps
- The protocol's temperature control method
- The runtime thermal data from the block and from the probe if it is installed

See "Printing a Program," chapter 6, for information about compatible printers.

Printing a Record of Actual Temperatures

A 0–5 VDC strip chart recorder may be used to print a graph of the temperature of the sample block over time, derived from the thermal sensor on the block.

To use the chart recorder, plug a miniature phone plug patch cord (3/32-inch twin conductor) into the chart recorder jack on the left side of the PTC-100 (fig. 2-2). Connect the other end to a chart recorder, being certain to observe correct polarity (the tip is positive).

Suitable chart recorders may be obtained from the following companies:

Gulton Graphic Instruments
1900 South County Trail
East Greenwich, RI 02818
Phone: 401-884-6800
Fax: 401-884-4872
Model: 288

Omega Engineering
PO Box 4047
Stamford, CT 06907
Phone: 800-826-6342
Fax: 203-359-7700
Model: RD288-5V-15IN

Kipp & Zonen
390 Central Avenue
Bohemia, NY 11716
Phone: 800-645-1025
Fax: 516-589-2068
Model: BD111

The Gulton and Omega machines have an accuracy of only ± 2% of span (i.e., less than ± 2°C), so they should only be used for event verification, not actual measurement. Kipp & Zonen's laboratory-grade flatbed machine has an accuracy of 0.3% of span, so it may be used for temperature measurement as well as event verification. However, calibration of output vs. temperature is still necessary. The Kipp & Zonen machine also has an optional-purchase feature allowing digital output of temperature data through a serial port, so that data may be downloaded into a computer.

6 Programming

The Elements of a Program

PTC-100 programs consist of a series of steps encoding a protocol. These steps are run using one of two temperature control methods: block control or probe control.

Programs may contain six types of steps. Two of the step types are mandatory, and four are optional:

1. **Temperature step (mandatory):** Sets a temperature for the block and the length of time it is held at that temperature. The PTC-100 brings the block to this temperature at its maximum rate of heating or cooling, unless modifying instructions are added to the program. (See chapter 2, "Specifications," for rates of heating and cooling for all block types.)
2. **GoTo step (optional):** Causes the program to cycle back to an earlier step for a specified number of times (up to 9,999 times).
3. **Increment step (optional):** Allows a progressive increase or decrease of temperature (–0.1° to 6.0°C/cycle) each time a step is executed in a GoTo cycle (useful in "touchdown" programs, when the optimal annealing temperature of an oligonucleotide is not known).
4. **Extend step (optional):** Allows a progressive lengthening or shortening of a temperature step hold (by 1–60 sec/cycle) each time a step is executed in a cycle (useful for accommodating an enzyme with diminishing activity).
5. **Slope step (optional):** Allows a slower-than-maximum rate of heating or cooling.
6. **End step (mandatory):** Instructs the PTC-100 to shut down its heat pump because the last line of the program has run.

Designing a New Program

Translating a Protocol into a Program

Until you are completely familiar with programming the PTC-100, you may find it helpful to first translate the protocol into program steps 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. 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 repeated 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 the denaturation and annealing/extension steps. 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]

Shortened 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)
4. End

Choosing a Temperature Control Method

The PTC-100 can control block temperature in two ways, each of which has different implications for the speed and accuracy of sample heating:

- **Block control:** The PTC-100 adjusts the block's temperature to maintain the **block** at programmed temperatures, independent of sample temperature.

- **Probe control:** The PTC-100 adjusts the block's temperature to maintain the **probe** at programmed temperatures.

Block Control

Block control is available for all models of the PTC-100. Under block control, the temperature of samples always lags behind the temperature of the block. The duration of this time lag depends on the sample vessel and sample volume but typically is between 10 and 30 seconds.

Probe Control

Probe control is available for PTC-100s equipped with the 60 block. Special care must be taken to fill the probe with the correct amount of oil and to seat the probe and the samples correctly. Otherwise, actual sample temperatures can vary widely from the probe's temperature. Probe control cannot be used with microplates or slides.

Under probe control, the machine will slightly overshoot its heat targets to account for the time required for heat to transfer to the sensor within the probe tube. Therefore, probe control protocols typically require incubations that are 15–20 seconds shorter and 1–2°C lower than incubations for block control protocols. For example, a denaturation step in a block-control protocol calling for 94°C for 30 sec would be reduced to 92°C for 10 sec in a probe-control protocol.

✔ **Tip:** Block control programs tend to be more trouble-free than probe control because fewer variables are involved in the temperature control feedback loop.

Entering a New Program

Programming the PTC-100 involves four steps:

1. Initiating the program
2. Naming the program
3. Entering the program's steps
4. Entering the End step

Each step involves typing in values from the keyboard or making selections from a menu. Programs may be edited as they are being entered or after they have been stored. Programs are automatically saved when the End step is entered.

Initiating the Program

To initiate a new program, select *Enter Program* from the Run-Enter Menu, then press «Proceed». The Main Menu will be displayed:

```
_NEW      List
 Edit     Delete
```

Select *New,* then press «Proceed». The naming screen will be displayed:

```
Name  A
```

Naming the Program

Program names may be up to eight characters long and may consist 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 enter the last character and again to enter 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.

Entering the Program Steps

When the name has been entered (CUSTOM1 is used in the following examples), the Enter Menu will be displayed:

```
Step 1      _TEMP
 Goto Option End
```

Use this menu to enter each step of the program:

- *Temp* enters a temperature step.
- *GoTo* enters a GoTo step.
- *Option* enters an increment, extend, or slope 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:

```
Step 1
Temperature _
```

The first line of this screen shows the number of the step being programmed (1 is used in the example above). The second line of the screen allows a target temperature (in degrees Celsius) to be entered for the step. Type any number between 0 and 100.0 as the target temperature (92.5 is used in the example below):

```
Step 1
Temperature 92.5
```

Press «Proceed». The temperature will be accepted, and a screen allowing entry of an incubation time will be displayed:

```
Step 1    Hrs _
Min      Sec
```

A value must be entered for *Hrs* (hours), *Min* (minutes), and Sec (seconds). Use the «Select» keys to move the cursor to each option, and either type a number or press «Proceed» to automatically enter a value of 0.

For example, to enter an incubation time of 30 seconds, press «Proceed» twice. The value of 0 will automatically be entered for *Hrs* and *Min*, and the cursor will be positioned at *Sec*. Use the keyboard to type "30." The screen will look like this:

```
Step 1    Hrs 0
Min 0    Sec 30_
```

Press «Proceed». The times will be accepted, and the Enter Menu will be displayed again. Use the Enter Menu to add another step to the program.

Entering a GoTo Step

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

```
Step 2
Go to step _
```

The first line of this screen shows the number of the step being programmed (2 is used in the example above). The second line of the screen allows entry of the number of the step the program should cycle back to.

Type the number of the step the program should cycle back to (1 is used in the example below):

```
Step 2
Go to step 1
```

Press «Proceed». The number will be accepted, and a screen allowing entry of an additional number of cycles will be displayed:

```
Step 2  Go to 1
_    more times
```

Type the additional number of times the program should cycle back to the step (24 is used in the example below):

```
Step 2  Go to 1
24    more times
```

Press «Proceed». The number will be accepted, and the Enter Menu will be displayed again. Use the Enter Menu to add another step to the program.

Entering an Increment Step

To enter an increment step, select *Option* from the Enter Menu and press «Proceed». The Options Menu will be displayed:

```
Step 3   _EXTEND
 Increment Slope
```

Select *Increment* and press «Proceed». A screen allowing entry of the initial incubation temperature for the step will be displayed:

```
Step 3
Temperature _
```

Type a temperature and press «Proceed». The number will be accepted, and a screen allowing entry of an incubation time will be displayed:

```
Step 3
Min _    Sec
```

Type a number for *Min* and *Sec*. Press «Proceed» to automatically enter a value of 0.

When the numbers have been typed, press «Proceed». The numbers will be accepted, and a screen allowing entry of a temperature increment or decrement will be displayed:

```
Step 3
Add _     °/cycle
```

Type a temperature from –0.1 to 6.0°C (the maximum programmable range) as the number of degrees by which the incubation temperature will increase or decrease each time the step is executed in a cycle. Increments as small as one tenth of a degree may be used. Use a negative number to program a progressive decrease of temperature, and a positive number to program a progressive increase.

When the number has been typed, press «Proceed». The number will be accepted, and the Enter Menu will be displayed again. Use the Enter Menu to add another step to the program.

Entering an Extend Step

To enter an extend step, select *Option* from the Enter Menu and press «Proceed». The Options Menu (see above) will be displayed. Select *Extend* and press «Proceed». Type the initial incubation temperature and incubation time as for an increment step (see above), and press «Proceed». The temperature and time will be accepted, and a screen allowing entry of a time increment or decrement will be displayed:

```
Step 4
Extend _  S/cyc
```

Type a number from –60 to 60 (the maximum programmable range) as the number of seconds by which the incubation time will increase or decrease each time the step is executed in a cycle. Use a negative number to program a progressive decrease of incubation time, and a positive number to program a progressive increase.

When the number has been typed, press «Proceed». The number will be accepted, and the Enter Menu will be displayed again. Use the Enter Menu to add another step to the program.

Entering a Slope Step

To enter a slope step, two values must be specified:

- **The desired temperature change for the step:** This is the difference between the temperature of the block at the beginning of the slope step and the desired block temperature at the end of the slope step. For example, if the final temperature of the preceding step is 92°C and the desired final temperature of the slope step is 65°C, the desired temperature change is 27°C.
- **The slope rate for the step:** This is a ratio of degrees per unit of time (usually degrees per second). Use the smallest convenient time increment to achieve the smoothest temperature curve for the step. For example, 0.1°C per second will give a smoother curve than 1°C per 10 seconds.

To enter a slope step, select *Option* from the Enter Menu and press «Proceed». The Options Menu (see above) will be displayed. Select *Slope* and press «Proceed». A screen allowing entry of the magnitude of the temperature change for the step will be displayed:

```
Step 4
Slope  _   °
```

Type a number for the desired temperature change and press «Proceed». The number will be accepted, and a screen allowing entry of the rate of heating or cooling will be displayed:

```
_  °per   Hrs
Min     Sec
```

Type a temperature from 0.1 to 1.5°C (the maximum programmable range) for the numerator of the slope rate and press «Proceed». The temperature will be accepted, and the cursor will move to *Hrs.* Type a time value for the denominator of the slope rate. A number must be entered for *Hrs, Min,* and *Sec*. Press «Proceed» to automatically enter a value of 0 and move the cursor to the next time element.

When the denominator has been typed, press «Proceed». The numbers will be accepted into the program, and the Enter Menu will be displayed again. Use the Enter Menu to add another step to the program.

Note: Not all blocks will be able to ramp at the maximum programmable speed of 1.5°/sec. See chapter 2, "Specifications," to determine the maximum speed possible for a given block.

Entering the End Step

To enter the End step, select *End* from the Enter Menu, then press «Proceed». A confirmation screen will be displayed:

```
Step 6
End
```

Press «Proceed». The End step will be entered into the program. The program will be stored, and the Run-Enter Menu will be displayed.

Programming an Indefinite Hold

Programs can be designed to end in an indefinite hold at a specified temperature. This allows completed reaction products to be held at a stable refrigeration temperature (often 4°C) at the end of a run, until they can be conveniently removed.

To program an indefinite hold, program a temperature step immediately before the protocol's End step. Type the desired temperature for the indefinite hold, then give the step an incubation time of 0 hours, 0 minutes, and 0 seconds. When this temperature step is reached, the block will heat or cool the samples to the desired temperature and hold them there until you manually end the program by pressing «Cancel» or «Stop».

Creating a Program That Requires Manual Stepping

To create a program that must be manually progressed to each step, program an indefinite hold for each temperature step in the protocol (see "Programming an Indefinite Hold," above). When the protocol runs, you may then advance it through its steps at the time desired.

Editing While Entering a Program

Changing the Last Value Entered or Last Menu Option Chosen

To change the last value entered or last menu option chosen, press «Cancel». The choice just made will be cancelled, and another value may be entered or another menu option chosen. **Press «Proceed» after changing a value, so that the program will accept it.**

Changing All Values in the Step Being Entered

To change all the values in the step currently being entered, repeatedly press «Cancel». Each time you press the key, the cursor will move backward through the step's values, deleting them 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 or re-enter the step.

Changing Values in Earlier Steps of a Program Being Entered

To change one or more values in earlier steps of the program you are entering, repeatedly press «Cancel» until the Enter Menu for the step you are working on is displayed. Press «Cancel» one more time to display the last-entered step. The step will be displayed in a special format:

```
CUSTOM1   3  94.0
   00:00:10
```

In the example above, the last-entered step is a temperature step. The step number is 3, and the temperature for the step is 94.0°C. The incubation time is displayed in the form of 00:00:00. The first two digits represent hours, the second two digits represent minutes, and the last two digits represent seconds. The step in the example is programmed for a 10-second incubation.

At this point you may use the arrow keys to scroll through the program's steps. To change temperature, time, and cycling values, see chapter 7.

Note: You cannot change the program name, step number, or step type. To change the program's name or the order or type of its steps, delete the program (see below) and then re-enter it.

After you have made all necessary changes, press the right «Select» key until the Enter menu is displayed for the next step in the program. Continue entering the new program.

Deleting an Incomplete Program

To delete a program that you are in the process of entering, enter the End step and press «Proceed», which will store the program. Then delete the program from memory (see below).

Deleting a Program from Memory

To delete a program, select *Enter Program* from the Run-Enter Menu and press «Proceed». The Main Menu will be displayed. Select *Delete* and press «Proceed». The deletion screen will be displayed for the first program in memory:

```
Delete
CUSTOM1?
```

Press the «Select» keys until the name of the program to be deleted is displayed. When the correct name is displayed, press «Proceed». The program will be deleted, and the Run-Enter Menu will be displayed.

Listing a Program

Use the *List* option on the Main Menu to display all of a program's steps in the LCD window. To enter List mode, select *Enter Program* from the Run-Enter Menu and press «Proceed». The Main Menu will be displayed. Select *List* and press «Proceed».

Press the «Select» keys until the name of the program to be listed is displayed, then press «Proceed». The first step in the program will be displayed in the LCD window. Press the right «Select» key to scroll forward and the left «Select» key to scroll backward through the list of steps. To exit List mode, scroll to the last step of the program, then press the right «Select» key once. The Run-Enter Menu will be displayed.

Note: No program values can be changed in List mode. To change program values, see chapter 7 .

Printing a Program

The PTC-100 can print all the programs stored in the machine. To do this, follow the instructions under "Listing a Program" to enter List mode. If a printer is connected to the printer port, the List program screen will give you the option to print as well as list programs in the LCD window:

```
_DISPLAY Program
 Print Programs
```

Select *Print Programs* and press «Proceed». Each program stored in the machine will be printed.

The PTC-100 parallel printer port is compatible with Epson® LX-810 dot matrix printers and many other IBM compatibles. Only printers that support the Epson extended graphics set will be able to print the degree character (°). Other printers will print an *x* instead of the degree character.

Keeping a Permanent Record of Programs

Occasionally in the course of repairing a PTC-100, 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 as described above or copied into a notebook.

7

Editing Programs

Initiating Editing

To edit an existing program the machine must be in Edit mode. To enter Edit mode, select *Enter Program* from the Run-Enter Menu and press «Proceed». The Main Menu will be displayed. Select *Edit* from the Main Menu and press «Proceed». A screen similar to the following will be displayed:

```
Edit
CUSTOM1?
```

The machine is now in Edit mode. Use the «Select» keys to select the program you wish to edit. When the name of the desired program is displayed, press «Proceed». The first step in the program will be displayed:

```
CUSTOM1  1  94.0
   00:00:20
```

The programmed temperature for this step is 94.0°C. The incubation time is displayed in the form of 00:00:00. The first two digits represent hours, the second two digits represent minutes, and the last two digits represent seconds. The step in the example is programmed for a 20-second incubation.

Editing the Program

Press the left «Select» key to scroll backward through the program steps, and the right «Select» key to scroll forward. As the left or right «Select» key is pressed, the cursor will progressively move backward or forward through each value in a step, and then back to the preceding or forward to the following step.

Editing Temperatures and Cycling Values

To change a temperature or a cycling value, position the cursor on it and type the new value. Press «Proceed». The new value will be accepted, and the cursor will move forward to the next value in the step.

To cancel a change, press «Cancel». The just-typed value will disappear, and a blank line will be displayed in its place. Type a new value and press «Proceed».

Note: **Be careful when editing program values.** Once a value is changed or deleted, you cannot make it reappear by pressing any key. To restore a value, you must retype it. You may find it helpful to write a value down before changing it, to minimize the chance that you will forget what it was.

Editing a Time Value

To edit any part of a time value, you must retype the number for each element in the time value: hours, minutes, and seconds. Press «Proceed» after typing each time element. The number just typed will be accepted, and the cursor will move to the next time element. To enter a value of 00 for hours, type a zero, then press «Proceed». The second zero will automatically be entered. To enter a value of 00 for minutes or seconds, simply press «Proceed». Both zeros will be entered at once.

For example, to change the time value for the example above from 00:00:20 to 00:00:30, press the right arrow key to move the cursor to the first digit of the time value:

```
CUSTOM1  1  94.0
   00:00:30
```

Type a zero. The zero will become the first digit of the hours element, and the values for minutes and seconds will disappear from the screen:

```
CUSTOM1  1  94.0
   0 :  :
```

Press «Proceed». A second zero will automatically be entered for the hours element, and the cursor will move to the minutes element:

```
CUSTOM1  1  94.0
   00:_ :
```

Press «Proceed». Two zeros will automatically be entered for the minutes element, and the cursor will move to the seconds element:

```
CUSTOM1  1  94.0
   00:00:_
```

Type the number 30, then press «Proceed». The number will be entered for the seconds element, and the cursor will move back to the first digit for the hours:

```
CUSTOM1  1  94.0
   00:00:30
```

Editing a Slope Step

To edit a slope step you must retype both a sign (plus or minus) and a number. Press «.» (the period key) to enter a plus sign and «-» (the hyphen) to enter a negative sign. Press «Proceed» after entering the new slope step value.

8 Maintenance

Cleaning the Chassis and Block

Clean the outside of the PTC-100 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 the block's wells whenever anything is spilled into them or when the oil in them is discolored or contains particulate matter. It is particularly important to prevent the buildup of old, dirty oil, which will interfere with vessel seating and diminish thermal coupling of sample vessels to the block.

Clean the wells with a swab moistened with water, 95% ethanol, or, if a thorough cleaning is needed, a 1:100 dilution in water of 5.25% (0.7M) sodium hypochlorite (household bleach). If using sodium hypochlorite, swab the wells with water afterward to remove all traces of it. Do not clean the block with caustic or strongly alkaline solutions (e.g., strong soaps, ammonia, sodium hypochlorite at a higher concentration than specified above). These can damage the block's protective anodized coating.

Note: See appendix A for information on cleaning the 16 MS Slide Block.

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. If these vents become clogged with dust and debris, airflow to the PTC-100's heat sink is hampered, eventually causing overheating and shutdowns (see "Air Supply Requirements," chapter 3).

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 methods, monitoring, and disposal of contaminated materials.

Changing the Fuses

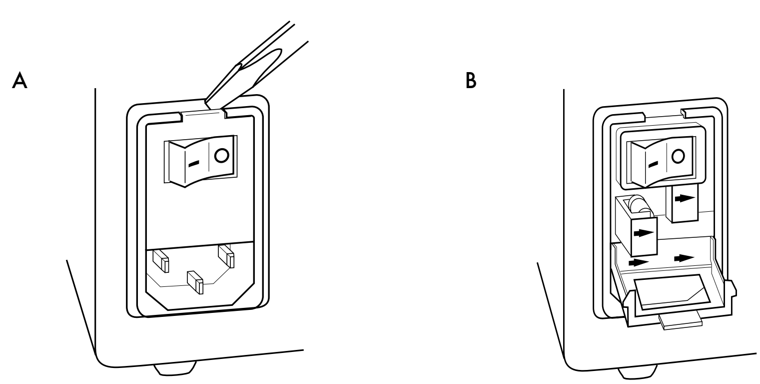
The circuits in the PTC-100 are protected by two fuses. When a fuse blows, the PTC-100 immediately shuts down and cannot be turned back on. The machine records the event as a power loss, so if a protocol is running when a fuse blows, the machine will resume the protocol run when the fuse is replaced and power restored (see "Resuming a Protocol after a Power Outage," chapter 5).

Warning: The PTC-100 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 PTC-100. You could receive a serious electrical shock. Opening the unit will also void your warranty.

Follow this procedure to change a fuse:

1. Move the power switch to the "0" (off) position. Disconnect the power cord from the back of the machine.
2. Insert one corner of a small flat-head screwdriver into the slot at the left side of the fuse block (fig. 8-1A). Gently pry the block loose, and pull it down and out. The fuses will be visible (fig. 8-1B).
3. Remove the fuses and examine them. A flat-head screwdriver may be used to pry the fuses loose, if necessary. A broken central wire or darkened glass indicates that a fuse has blown and should be replaced.
4. Gently press the fuse block back into place, and reconnect the power cord.

Figure 8-1 Changing a fuse.



9 Troubleshooting

Error Messages

Error Message	Cause	Action
Check Block Sensor	Block's temperature sensor or sensor circuitry is malfunctioning.	Block may need servicing. Contact MJ RESEARCH or your distributor.
Heat Pump Not Functioning	Heat pump is not working, so machine has shut down.	Base needs servicing. Contact MJ RESEARCH or your local distributor.
HS Overheating, Check Air Flow	Machine is not getting enough air, or air being taken in is not cool enough.	Ensure machine is at least 10cm away from walls and other thermal cyclers. Make sure air being taken in is cool (see chap. 3). If message persists, base may need servicing; contact MJ RESEARCH or your distributor.
Memory Full	Memory has reached its capacity of 1600 steps among all stored programs.	Delete programs as necessary to free memory.
Missing Calibration	A memory failure has caused machine to lose block calibration.	Contact MJ RESEARCH or your distributor.
Missing Probe Calibration	Follow correct probe calibration procedure (see p. 4-12).	Turn machine off, then on again, and run protocol again. If message persists, contact MJ RESEARCH or your distributor.
Probe Input Not Valid	Probe needs recalibration or is defective.	Recalibrate probe (see p. 4-12). If message persists, probe may need replacing. Contact MJ RESEARCH or your distributor.
Service Heat Pump Soon	Heat pump is malfunctioning and need servicing.	Base needs servicing soon, before heat pump stops working completely. Contact MJ RESEARCH or your distributor.
Service Heat Pump Now	Heat pump needs replacing.	Contact MJ RESEARCH or your distributor.

Problems Related to Protocols

Problem	Cause	Action
Reaction is working but broad low mole-cular weight band is seen in gels.	"Primer-dimer" material often produces a broad band in the <100bp region of gels.	If obtaining appropriate reac-tion product/s, no need to change anything.
		Minimize "primer-dimer" production by designing primers with no 3' self-complementarity.
		Reoptimize magnesium concen-tration and annealing temper-ature to maximize desired pro-duct and minimize "primer-dimers."
Reaction working but unexpected extra products or smear is seen.	Nonspecific hybridization occur-ring during setup.	Program a hot start into the protocol.
	Reaction component concentration too high or too low.	Check concentrations of compo-nents. May need to reoptimize magnesium concentration.
	Annealing temperature too low.	Reoptimize annealing temperature.
	Protocol contains a wrong value.	Use List to check protocol's temperature control method, temperatures, and times.
	Template not of sufficient purity.	Check extraction and purifi-cation protocols. Add additional purification steps if necessary.
	Multiple templates or host DNA in sequencing reactions.	Check nucleic acid preparations by gel electrophoresis.

(Continued)

Problem	Cause	Action
No reaction products obtained.	Wrong protocol used.	Re-run reaction using correct protocol.
	Protocol contains a wrong value.	Use List to check protocol's temperature control method, temperatures, and times.
	Reaction component omitted from mixture.	Check reaction assembly proto-col, ensuring that mixture con-tains appropriate components in correct concentrations.
	Denaturation temperature too low.	Use 92° C for denaturation.
	Annealing temperature too high for primers.	Check for appropriate anneal-ing temperatures of primers, using available computer pro-grams or empirical testing.
	Wrong temperature control method used.	Use List to check temperature control method for protocol; change if needed.
	Probe failed, causing machine to run protocol under block control.	Check screen for probe failure error message. Probe may need servicing or replacing. Call MJ RESEARCH or your local distri-butor.
	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 Machine Performance

Problem	Cause	Action
Beeper sounds continu-ously (PTC-100 with Hot Bonnet only).	Hot Bonnet has been enabled, but it is not plugged into the machine.	Cancel protocol. Plug Hot Bon-net cable into its port at back of machine, and restart protocol.
Block does not heat or cool. LED bar graph is all red or all green.	Heat pump is malfunctioning.	Contact MJ RESEARCH or your distributor.
Machine will not power up.	Power cable is not plugged into machine, electrical outlet is dead, or fuse has blown	Make sure power cord is plugged into machine correctly. Make sure electrical outlet is operational. Check fuse.
Machine starts in middle of a protocol when turned on.	Machine was turned off before protocol run had finished.	See p. 5-5.
Strange characters appear in LCD window, or text in window freezes or scrolls repe-titively.	LCD window or machine microprocessor is malfunc-tioning.	Reset the microprocessor by turning machine off, then back on while pressing the Cancel key at the same time. If problem persists, contact MJ RESEARCH or your distributor.

Appendix A

The 16MS Slide Block

Description of the 16MS Slide Block

The 16MS Slide Block can thermally cycle slides and tubes. The block consists of a vertical metal rack holding up to 16 standard 25 x 75mm slides (see fig. 2-6). Four removable thin plastic rods are inserted into holes drilled along the back of the rack. These rods prevent loaded slides from slipping out the back side of the block. Slides heat and cool uniformly as long as there is complete contact between their bottoms and the metal slots and the block's lid is in place.

The block can also hold 24 x 0.2ml tubes, in holes drilled into the block. This feature is useful for optimizing primers and temperatures and running control reactions for reactions run on slides.

A wide variety of reactions done on slides may be run in the 16MS Slide Block, such as PRINS, RT-PRINS, *in situ* PCR[*] and RT-PCR, and immunohistochemical procedures. Using Frame-Seal incubation chambers to control evaporation, the slide block also may be used for precise thermal control of *in situ* hybridizations.

Operating the 16MS Slide Block

Selecting the Correct Sample Vessel

Suitable slides can be made of any thermally conductive material and be any shape as long as the finished slide fits in the block's slots. See "Selecting the Correct Sample Vessel," chapter 4, to select tubes.

Preparing and Sealing Sample Vessels

Slides

To ensure that all samples reach the same temperatures during thermal cycling, the bottoms of loaded slides must be in complete contact with the slots that they rest on. Do not use any slide preparation method that would interfere with this, such as clips that extend under the slide.

Do not use mineral oil under the slides to enhance thermal contact. This is unnecessary, and the oil is difficult to completely clean off, eventually making the slots sticky.

Use special care in sealing cover glasses or the edges of two-slide preparations (i.e., where a slide is being used as a cover glass) with such sealers as nail polish or rubber cement. Allow the sealer to completely dry before loading slides into slots, to avoid getting sealer on the surface of the slots (especially a problem with two-slide preparations). Keep the bottoms of slides free of sealer, to avoid interposing anything between the slides and the bottom of the slide slots.

Self-Seal reagent and Frame-Seal incubation chambers, available from MJ Research, are two convenient and effective alternatives to messy and time-consuming slide sealing methods. Other approaches to slide sealing are possible; contact MJ Research for more information.

Tubes

Since the 16MS Slide Block does not have a heated lid, tube reactions require an oil overlay.

Loading Sample Vessels into the Block

Slides

Follow this procedure to load slides into the 16MS Slide Block:

1. Inspect the block to ensure that its slots are clean and free of oil (see below for cleaning instructions).
2. Close off the back end of the block by inserting the small plastic rods into their holes (see fig. 2-6). This will keep the slides within their slots during loading.
3. Place slides in slots. Avoid dislodging unsealed cover glasses or the top slides in two-slide preparations. Using a forceps can make it easier to load slides

   Make sure all slides are lying flat in their slots. Remove slides that are not lying flat, clean off or remove whatever is interfering with the slides' seating, and re-load the slides into the block.
4. Place the removable lid over the block, to ensure that samples heat and cool uniformly.

Tubes

See "Loading Sample Vessels," chapter 4, for instructions on loading and thermally coupling tubes to the 16MS Slide Block. Place the removable lid over the block after tubes have been loaded, to ensure even heating and cooling of the tubes.

Programming the 16MS Slide Block

When designing protocols for the 16MS Slide Block, remember that temperature steps must be long enough to allow for the time required to heat the slide from the bottom to the top, where the reactants lie. All temperature steps should be made at least 20 seconds long for all new protocols. This length of time is sufficient to heat a typical glass slide. Once a protocol has been confirmed to work, you may empirically test shorter temperature steps.

All programs for the 16MS Slide Block must be run under block control, as a probe is not available for this machine.

Maintaining the 16MS Slide Block

Clean the 16MS Slide Block with a mild soap solution, water-based laboratory cleaner, or ethanol (95%) and a soft cloth. Avoid strongly alkaline cleaners and chlorinated solvents. Do not immerse the block in water.

Remove extraneous materials from slide slots with a long-handled cotton swab, small glassware cleaning brush, or tongue depressor wrapped in laboratory tissue. Pay particular attention to cleaning the bottoms of slots. Remove nail polish from slots with acetone.

Appendix B

Safety Warnings

Warning: Operating the PTC-100 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 machine.

Warning: Do not open or attempt to repair the PTC-100 or any accessory to the PTC-100. Doing so will void your warranties and can put you at risk for electrical shock. Return the PTC-100 to the factory (US customers) or an authorized distributor (all other customers) if repairs are needed.

Warning: The PTC-100 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.

Warning: The PTC-100 incorporates neutral fusing, which means that live power may still be available inside the unit even when the fuse has blown or been removed. Never open the PTC-100; you could receive a serious electrical shock. Opening the unit will also void your warranties.

Safe Use Guidelines

The PTC-100 is designed to be safe to operate under the following conditions:

- Indoor use
- Altitude up to 2000m
- Ambient temperature 4–32°C
- Humidity 10–90%, noncondensing
- Transient overvoltage per Installation Category II, IEC 664
- Pollution degree 2, in accordance with IEC 664
- Installation category II

Electromagnetic Interference

The PTC-100 has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the US FCC Rules. These limits are designed to provide a reasonable protection against harmful interference when the equipment is operated in a commercial environment. This machine 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 machine in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his or her own expense.

In addition, the PTC-100 design has been tested and found to comply with the EMC standards for emissions and susceptibility established by the European Union at time of manufacture.

FCC Warning

Changes or modifications to the PTC-100 not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

Appendix C

Factory-Installed Protocols

Name	Step	Temperature & Time
ICEBUKET	1	4° , 0:00:00
LIGATION	1	15° , 0:00:00
37° -1HR	1	37° , 1:00:00
	2	4° , 0:00:00
37° -6HR	1	37° , 6:00:00
	2	4° , 0:00:00
65°	1	65° , 0:00:00
DENATURE	1	95° , 0:05:00
BOIL	1	100° , 0:05:00
CUT&KILL	1	37° , 1:00:00
	2	70° , 0:05:00

Appendix D

Warranties

U.S. & Canadian Warranty, Standard

MJ Research, Incorporated warrants new MJ Research brand thermal cyclers (Models PTC-100, PTC-150, PTC-200 & PTC-225) against defects in material and workmanship for a period of two years from the date of purchase. If a defect is discovered, MJ Research, Incorporated will, at its option, repair, replace, or refund the purchase price of the thermal cycler at no charge to the customer, provided the product is returned to MJ Research, Incorporated within the warranty period. In no event will MJ Research, Incorporated be responsible for damage resulting from accident, abuse, misuses, or inadequate packaging of returned goods.

Any implied warranties, including implied warranties of the merchantability and fitness for a particular purpose, are limited in duration to two years from the date of original retail purchase of this product.

The warranty and remedies set forth above are exclusive and in lieu of all others, oral or written, expressed or implied. No MJ Research dealer, agent, or employee is authorized to make any modification, addition, or extension to this warranty, except in the form of the extended warranty outlined below.

MJ Research, Incorporated is not responsible for special, incidental, or consequential damages resulting from any breach of warranty, or under any other legal theory, including downtime, lost samples or experiments, lost reagents, lost profits, goodwill, damage to or replacement of equipment, property, and any costs of recovering or reproducing experimental results and data.

Exclusions: This warranty applies only to machines sold in the U.S.A. and Canada. Under no circumstance will MJ Research, Incorporated ship a repaired or replaced machine, or grant a refund of purchase price, to a user in a nation in which there was an authorized MJ Research distributor at the time of purchase. This warranty is not transferable from the original purchaser to a subsequent owner. Furthermore, this warranty does not apply to instruments used outside the U.S.A. and Canada, except when expressly authorized in writing by MJ Research, Incorporated.

U.S. & Canadian Extended Warranty, Optional

MJ Research, Incorporated will offer to each original purchaser of an MJ Research brand thermal cycler the opportunity to purchase an extension of the warranty coverage explained above for an additional two years. The coverage must be purchased through a purchase order received by MJ Research, Incorporated within 30 days of receipt of the offer of extended warranty or the offer to renew the extended warranty. These offers apply only to machines sold and used in the U.S.A. and Canada.

Some states of the U.S.A. do not allow the exclusion or limitation of incidental or consequential damages or limitations on how long an implied warranty lasts, so the above limitation or exclusion may not apply to you. This warranty gives you specific legal rights. You may also have other rights depending upon your state of residence.

All provisions of this warranty are voided if the product is resold, repaired, or modified by anyone other than MJ Research, Incorporated or an authorized distributor.

Appendix E

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 number 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.
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

Index

M

O

P

R

S

MJ Research, Inc.

The Originator of Peltier Thermal Cyclers

DECLARATION OF CONFORMITY

(for attachment of "CE" mark, as well as to document Canadian & US compliance)

MJ RESEARCH, INCORPORATED, the manufacturer of the PTC-100™ thermal cycler, hereby declares that the instrument conforms to the following:

APPLICATION OF E.U. COUNCIL DIRECTIVES: 89/336/EEC & 73/23/EEC

STANDARDS TO WHICH CONFORMITY IS DECLARED:

EU: **IEC 61010-1,** Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I, including Amendments 1 & 2

EU: **EN61326:1998, Annex B,** Electrical Equipment for Measurement, Control, and Laboratory Use, EMC Requirements, including Radiated & Conducted Emissions, and Immunity

CANADA: **CAN/CSA-C22.2 No. 1010.1-92 & CAN/CSA-C22.2 No. 1010.1B-97,** Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part I, including Amendments 1 & 2

US: **Underwriters Laboratories (UL) Std. No. 3101.1,** Electrical Equipment for Laboratory Use, Part I

TYPE OF EQUIPMENT: European Union Class "D"; Electrical Equipment for Measurement, Control, and Laboratory Use

MODEL NUMBER: Model "PTC-100™" thermal cycler, rated 100-240 VAC, 50-60Hz, 350 W (momentary 4.5 A). CSA International certifies as, "Equipment Class I, Pollution Degree 2, Installation Category II".

YEARS OF MANUFACTURE: 6 September 2001 and onward

MJ RESEARCH INCORPORATED, manufacturer of the equipment described above, certifies this model of instrument has been tested and conforms to the applicable Directives & Standards of the European Union (EU), as well as those for Canadian and US compliance, as described above.

Test data to verify this conformity are available for inspection at **MJ RESEARCH, INCORPORATED,** both at the U.S. manufactory detailed below, as well as at the MJ Research European Representative Office at Kirke Værløsevej 16; 3500 Værløse, Denmark (+45) 44 35 05 40, fax (+45) 44 35 05 49.

9/20/2001
Date signed

Robert A. Iovanni
Electrical Engineer, on behalf
of MJ RESEARCH, INCORPORATED
590 Lincoln Street
Waltham, Massachusetts 02451 USA
tel (617) 923-8000 • fax (617) 923-8080

1
Copy Number