PTC-100® Thermal Cycler Operations Manual

Version 9.0

(applicable to revision 6 instruments only)

BIO·RAD

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Operations Manual

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BIO-RAD

NOTICE TO PURCHASER

Purchase of this instrument, Serial No. _____, conveys a limited non-transferable immunity from suit for the purchaser's own internal research and development and for use in applied fields other than Human In Vitro Diagnostics under one or more of U.S. Patents Nos. 5,656,493, 5,333,675, 5,475,610 (claims 1, 44, 158, 160-163 and 167 only), and 6,703,236 (claims 1-7 only), or corresponding claims in their non-U.S. counterparts, owned by Applera Corporation. No right is conveyed expressly, by implication or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5' nuclease methods. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

07512 rev F

Table of Contents

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 PTC-100 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 this machine.

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

Warning: The PTC-100 cycler 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 cycler 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 cycler base; you could receive a serious electrical shock. Opening the unit will also void your warranties.

Safe Use Guidelines

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

- Indoor use

- Altitude up to 2000 m

- 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

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 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 keypad keys are 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 four lines of text:

Example:

```
PTC-100:
_RUN        Enter
List        Edit
Files       Setup
```

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 Cycler

Thank you for purchasing a Bio-Rad PTC-100® thermal cycler. Designed by a team of molecular biologists and engineers, the PTC-100 cycler will meet your needs for a reliable, easy-to-use, programmable thermal cycler. The latest (revision 6) PTC-100 cycler features:

• Expanded four-line LCD display for quick programming, editing, and file management

• Updated software with more editing and file management options including the ability to store programs in password-protected folders

• A standard Hot Bonnet® heated lid for oil-free cycling

• Space-saving design for easy setup and transportation

• Instant Incubate feature for continuous-temperature incubations

How to Use This Manual

This manual contains all the information you need to operate the revision 6 PTC-100 cycler safely and productively. Revision 6 instruments can be distinguished by the presence of the blue LED power light (see figure 2-3), and the four-line LCD window. For older model PTC-100 cyclers, please refer to the PTC-100 cycler *Operations Manual* V8.1, which can be found at **discover.bio-rad.com**.

• Chapter 2 describes the **physical characteristics and specifications** of the PTC-100 cycler including the updated layout of the control panel.

• Chapters 3 and 4 describe **installing and operating** the PTC-100 cycler.

• Chapters 5-8 describe **programming** the PTC-100 cycler. Chapter 5 describes how to run an existing program including how to locate programs stored in the main folder or in custom folders. Chapter 6 chronicles program creation. Note the replacement of the slope option with the new ramp step, and the added beep option which can be used to signal step completion. Chapter 7 provides instructions for editing programs including adding and deleting both steps and options from a program. Chapter 8 describes utilities for creating, deleting, and password protecting folders, as well as for copying, renaming, moving, and deleting programs.

• Chapter 9 describes proper **maintenance** of the PTC-100 cycler.

• Chapter 10 offers advice on **troubleshooting** the PTC-100 cycler.

Important Safety Information

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

⚠ **Warning:** The PTC-100 cycler 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 beginning of this manual on pages iv and v and exercise all precautions outlined in them.
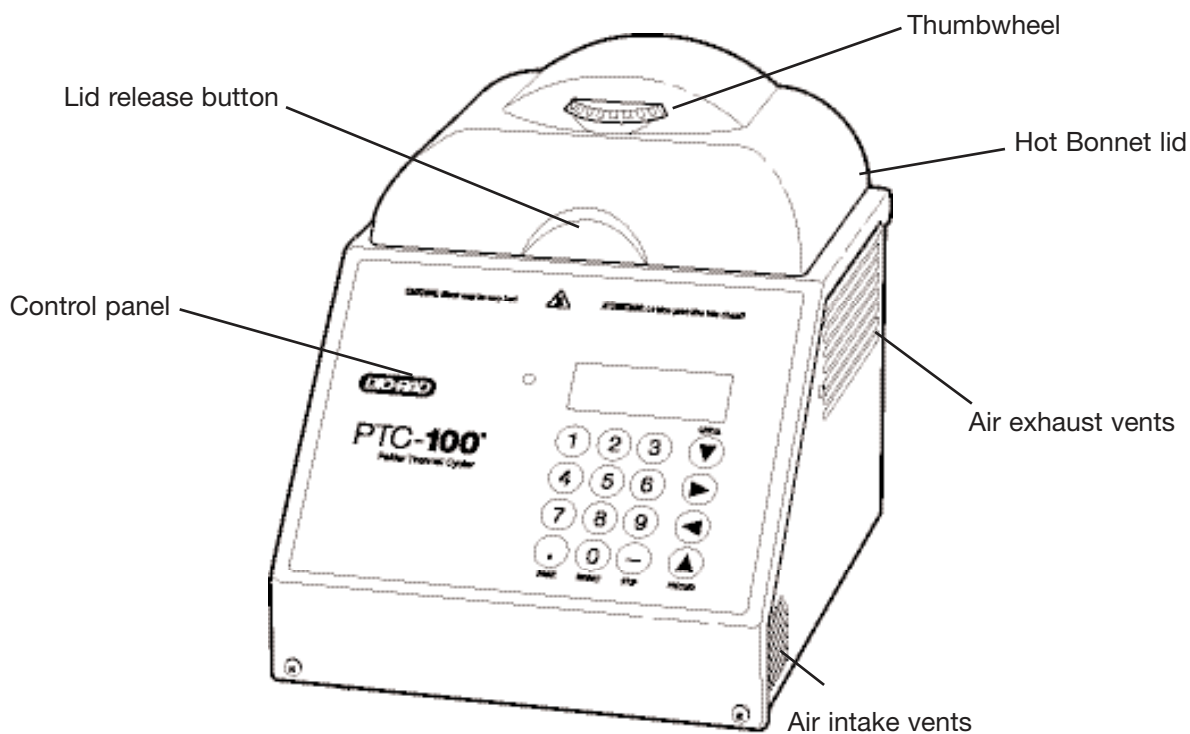
2 Layout and Specifications

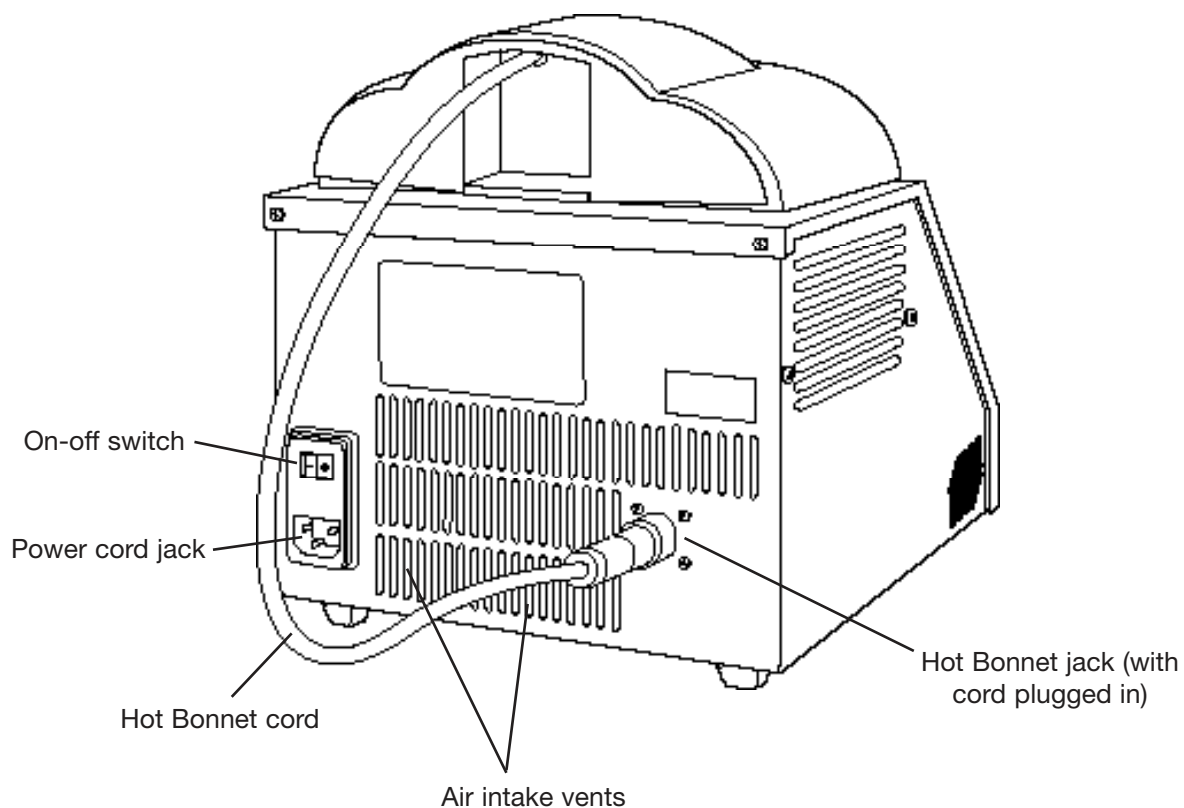# PTC-100 Cycler with Hot Bonnet Lid

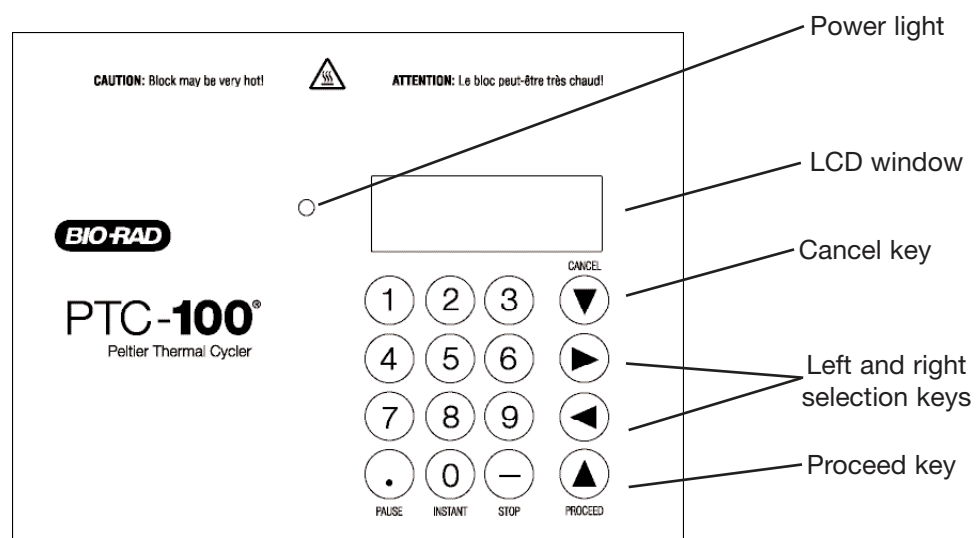## Models Available

60-well block: holds 60 x 0.5 ml tubes

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

Front View (Fig. 2-1)

Back View (Fig. 2-2)

On-off switch

Power cord jack

Hot Bonnet cord

Air intake vents

Hot Bonnet jack (with cord plugged in)

Control Panel (Fig. 2-3)

Power light

LCD window

Cancel key

Left and right selection keys

Proceed key

Accessories

Slide Griddle Adaptor

The Slide Griddle™ adaptor allows the PTC-100 cycler to thermally cycle up to four 25 x 75 mm glass slides. The adaptor is available for 60-well and 96-well models of the PTC-100 cycler.

Specifications

Thermal range:	0–100°C
Accuracy:	±0.5°C of programmed target at 60°C
Thermal uniformity:	±0.4°C well-to-well within 30 sec of arrival at 60°C
Ramping rate:	60-well block: up to 1°C/sec 96-well: up to 1.2°C/sec
Sample capacity:	60-well block: 60 x 0.5 ml tubes 96-well block: 96 x 0.2 ml tubes or one 96-well plate
Line voltage:	100–240 VAC rms (no adjustment needed among voltages within these ranges)
Frequency:	50/60 Hz single phase
Power:	350 W maximum (momentary 4.5 A)
Fuses:	Two 4 A, 5 x 20 mm (4 ampere)
Displays:	One 4 x 20 LCD alphanumeric display
Memory:	99 typical programs in up to 12 individual folders
Weight:	7.0kg
Dimensions:	PTC-100 cycler with Hot Bonnet lid, 23 x 28 x 26 cm

3 Installation

Packing List

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

- One PTC-100 cycler, with Hot Bonnet® lid
- One power cord
- Two spare fuses
- *The PTC-100 Thermal Cycler Operations Manual* (this document)

If any of these components are missing or damaged, contact Bio-Rad or the authorized distributor from whom you purchased the PTC-100 cycler to obtain a replacement. Please save the original packing materials in case you need to return the PTC-100 cycler for service. See Appendix B for warranty information.

Setting Up the PTC-100 Cycler

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

Screw the Hot Bonnet lid's power cord into its jack at the back of the instrument (see fig. 2-2; finger-tightness is adequate).

Situate the instrument according to the instructions below.

Environmental Requirements

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

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

Power Supply Requirements

The PTC-100 cycler requires 100–240VAC, 50–60Hz, 350W (4.5 amps momentary), and a grounded outlet. The instrument can use current in the specified range without adjustment, so there is no voltage-setting switch.

The PTC-100 cycler is equipped with a power-entry module that accepts cordsets with an IEC 60320–1 type C13 connector (this is the same standard configuration used by many computer manufacturers for their equipment). All cordsets used with the PTC-100 cycler must be rated to carry at least 10A at 125V or 250V, the latter specification depending upon the supply voltage used. Additionally, the cordset must meet all other applicable national standards—thus at a minimum, the cordset 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

The PTC-100 cycler requires a constant supply of air that is 32°C or cooler in order to remove heat from the instrument's heat sink. Air is taken in from vents at the rear, sides, and bottom of the instrument and exhausted from vents on both sides (see figures 2-1 and 2-2). If the air supply is inadequate or too warm, the instrument can overheat, causing performance problems, software error messages, and even automatic shut-downs.

Ensuring an Adequate Air Supply

- Do not block the air-intake vents.

  Position the PTC-100 cycler at least 10cm from vertical surfaces and other thermal cyclers (greater distances may be required; see below). Do not put loose papers, bench paper, or this manual under the instrument; they can be sucked into the air-intake vents on the bottom.

- 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 instrument off prior to cleaning or vacuuming air vents.

Ensuring That Air Is Cool Enough

- Do not position two or more PTC-100 cyclers (or other thermal cyclers) so that the hot exhaust air of one blows directly into the air-intake vents of another.

- Make sure the PTC-100 cycler receives air that is 32°C or cooler by measuring the temperature of air entering the instrument through its air-intake vents.

  Place the PTC-100 cycler where you plan to use it, and turn it on. Try to reproduce what will be typical operating conditions for the instrument 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 PTC-100 cycler, then measure the air temperature at the back 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 32°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.

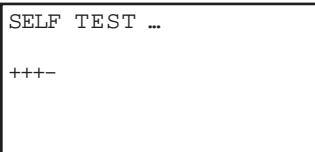**Table 3-1** Troubleshooting Air Supply Problems

Cause	Possible Remedies
Air circulation is poor	Provide more space around 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 instruments so that warm exhaust air does not enter intake vents

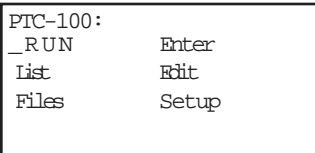# 4 Operation

Turning the PTC-100 Cycler On

Move the power switch, located at the right rear of the instrument, to "1" (the "On" position). The fan will turn on, and the power light on the control panel will glow blue. A self test of the heat pumps will begin running. Three heating (+) and one cooling test (-) will be performed. Test progress is tracked on a screen in the LCD window:

```
SELF TEST …

+++-
```

This screen disappears within 20 seconds. If a problem is detected, the display shows an error message.

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

```
PTC-100:
_RUN         Enter
 List        Edit
 Files       Setup
```

The PTC-100 cycler is now ready to execute programs.

Understanding the Main Menu

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

- **Run:** Executes a program.

- **Enter:** Allows new programs to be entered.

- **List:** Displays a program's steps.

- **Edit:** Allows modification of stored programs.

- **Files:** Accesses file management utilities.

- **Setup:** Accesses the heated lid settings, as well as, the instrument's software version and serial number.

Using the Control Panel

The control panel (see fig. 2-3) includes operation keys, an LCD window for displaying programming and instrument 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»:** Initiates an Instant Incubation program that sets up the PTC-100 cycler as a constant-temperature incubator.

Indicator Light

- **Power light:** Glows blue when the PTC-100 cycler is powered up.

Opening and Closing the Lid

Opening the PTC-100 cycler with Hot Bonnet lid: Push on the button at the lid's center and lift the lid (see fig. 4-1).
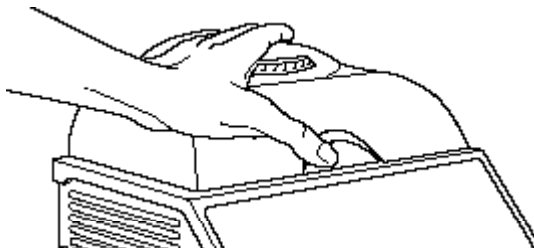
Closing all PTC-100 cycler models: Push the lid down to cover the block. Make sure that lid pressure is correctly adjusted by using the thumbwheel as described in the "Adjusting the Hot Bonnet Lid's Pressure" section in this chapter.

⚠ **Caution:** Do not use excessive force to close the lid, as this may damage the lid mechanism.

(Fig. 4-1)

Selecting the Correct Sample Vessel

Bio-Rad offers a full range of tubes and microplates, manufactured to the specifications of each type of block to ensure a precise fit. See Appendix 4-A of this chapter 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.

0.5 ml Tubes

Thick-walled 0.5 ml tubes may not fit tightly in thermal cycler wells and typically provide poor thermal transfer, since these tubes were originally designed for centrifuges (see the "Using Oil to Improve Thermal Contact" section in this chapter). For best results, we recommend using thin-walled 0.5 ml tubes specifically designed for thermal cycling. The higher quality brands provide a good and consistent fit. Bio-Rad thin-walled 0.5 ml tubes are designed for precise block fit and tight sealing of reactions down to 10 µl.

0.2 ml Tubes

All types of thin-walled 0.2 ml tubes may be used. Bio-Rad offers high-quality 0.2 ml tubes in a number of styles, including individual and strip tubes.

Microplates

A variety of polycarbonate or polypropylene microplates can 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 (see "Safety Warning Regarding Use of ^{35}S Nucleotides" in chapter Appendix 4-B. This allows smaller sample volumes to be used—as little as 5–10 µl.

Several varieties of microplates are available from Bio-Rad (see the "Tube, Microplate, and Sealing Selection Chart" in chapter Appendix 4-A).

Sealing Sample Vessels

To avoid changing the concentration of reactants, steps must be taken to prevent the evaporation of water from reaction mixtures during thermal cycling. Only a layer of oil or wax will completely prevent evaporation from the surface of the reaction fluid. However, an adequate degree of protection can be achieved by sealing vessels with caps, film, adhesive seals, or mats, then cycling the samples using the heated lid to prevent condensation at the tops of tubes.

Sealing with Oil or Wax

Mineral oil, silicone oil, paraffin wax, or Chill-out™ liquid wax may be used to seal samples. Use only a small amount of oil or wax; 1–3 drops (15–50 µl) are usually sufficient. **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 Bio-Rad) 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 tip can then be used to pierce the wax in the tubes and remove the samples. The wax is available in a clear, optical-assay grade or dyed red to assist in monitoring its use. The red dye has no adverse effects on fluorescent gel analysis of reaction products.

Sealing with the Hot Bonnet Lid

The Hot Bonnet heated 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 and lid, so that solution concentrations are unchanged by thermal cycling. The Hot Bonnet lid 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, adhesive seals, or mats must be used along with the Hot Bonnet lid 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).

Note: 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 complete.

Microseal® 'A' film offers a quick alternative to sealing microplates or arrays of tube strips. This film is specially designed to seal tightly during cycling, yet release smoothly to minimize the risk of aerosol formation and cross-contamination of samples. Microseal 'A' film is easily cut for use with fewer than 96 samples.

Microseal 'B' adhesive seals feature an aggressive adhesive, effective from –20°C to 110°C, which allows secure sample storage or transport before and after cycling. The clear polyester backing allows easy inspection of sample wells. Microseal 'B' clear, adhesive seals are ideal for thermal cycling in all polypropylene and polystyrene microplates.

PCR plate sealing mats (223-9442) are an economical means to seal 96-well microplates. An array of 96 bumps on the mat helps orient it on the microplate and prevents the mat from sticking to the heated lid. The mats may be cleaned with sodium hypochlorite (bleach) for reuse, and they are autoclavable.

Adjusting the Hot Bonnet Lid's Pressure

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

Follow these steps to adjust the pressure exerted by the inner lid:

1. Make sure the block's wells are clean. Even tiny amounts of extraneous material can decrease thermal conduction and interfere with the proper seating of a microplate or tubes.

2. Open the Hot Bonnet lid. Turn the 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 lid by pressing down on the top. Turn the thumbwheel clockwise to lower the 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. For microplate sealing films or mats that require additional pressure, first open the Hot Bonnet lid, then turn the thumbwheel clockwise an extra half turn past the point of initial contact 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 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 lid. 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.

⚠ **Caution:** Do not use excessive force to close the lid, as this may damage the lid mechanism.

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 lid, also load at least one empty tube in each corner of the block, to ensure that the lid exerts even pressure on the tubes (see the "Adjusting the Hot Bonnet Lid's Pressure" section above).

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 9 for cleaning instructions).

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

Using Oil to Improve Thermal Contact

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

• Oil traps dirt, which interferes with thermal contact between vessels and the block.

• If used in excess of recommended amounts, oil can seep into the instrument and damage internal components.

• Oil 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.

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

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).

Using the Slide Griddle™ Adaptor

The Slide Griddle adaptor heats up to four glass slides for *in situ* protocols. The Slide Griddle adaptor may be used with PTC-100 cyclers that have the 96-well block or the 60-well block. See the *Slide Griddle Operations Manual* and *Passive Slide Griddles* for *Bio-Rad Thermal Cyclers* for instructions on operating the Slide Griddle adaptor.

Appendix 4-A

Tube, Microplate, and Sealing System Selection Chart

The following sample vessels and sealing options are recommended for use with the PTC-100 cycler and are available from Bio-Rad.

To place an order, call 1-800-4BIORAD.

Key

✔ Reaction vessel fits block/sealing option fits reaction vessel without modification.

✁ Reaction vessel/sealing option can be cut to fit.

PTC-100 Thermal Cycler Blocks		Reaction Vessels		Sealing Options for Oil-Free Cycling					
96 (0.2 ml)	60 (0.5 ml)	Description	Bio-Rad Catalog #	Microseal 'A' film MSA-5001	Microseal 'B' seal MSB-1001	Sealing mat 223-9442	8-Cap Strips TCS-series	12-Cap Strips TCS-series	Chill-out wax CHO-series
✔		Hard-Shell skirted 96-well PCR plates	HSP-series	✔	✔	✔	✔	✔	✔
✔		Microseal skirted 96-well PCR plates	MSP-series	✔	✔	✔	✔	✔	✔
✔		Multiplate™ unskirted 96-well PCR plates	MLP-series MLL-series	✔	✔	✔	✔	✔	✔
✔		Multiplate unskirted 48-well PCR plates	MLP-series MLL-series	✁	✁	✁	✔	✔	✔
✔		Multiplate unskirted 24-well PCR plates	MLP-2401	✁	✁	✁	✔	✁	✔
✔		Concord™ skirted 96-well PCR plates*	CON-9601	✔					✔
✔		0.2 ml 8- and 12-tube strips	TBS-series TLS-series	✔			✔	✁	✔
✔		0.2 ml individual tubes without caps	TBI-series	✔			✔	✔	✔
✔		0.2 ml individual tubes with caps	TFI-0201 TWI-0201						✔
	✔	0.5 ml individual tubes with caps	TBI-series						✔

Note: Concord microplates are made from polycarbonate plastic, which is more prone to poor sealing and vapor leakage during stringent thermal cycling.

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 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.

0.5 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 0.5ml 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 occasionally and carefully. 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 (such as those above) are corrosive and must be *thoroughly* rinsed away within a few minutes of application. They can eat away the surface finish of the blocks.

5 Running Protocols

Running a Protocol

Either a custom-designed protocol or one of the factory-installed resident protocols may be run. See Appendix A 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 two or more screens listing the protocols will be displayed. Custom protocols are listed first, then the factory-installed programs:

  ```
  Run        (<MAIN>)
  _CUSTOM1  CUSTOM2
  ICEBUKET  LIGATION
  37 -1HR   37 -6HR
  ```

  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». If the heated lid is attached, a screen will appear asking if the heated lid should be used.

  ```
  Run: CUSTOM1
  Use Heated Lid?
    yes       _NO
  ```

  Select yes to use the heated lid at the target temperature specified in the Setup Menu (see Chapter 8), or select no to apply no heat to the lid. Press «Proceed», and the protocol will begin running.

Reading the Runtime Screen

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

```
Run: CUSTOM1
1= 95.0  for 5:00
Cycle: 1
Block: 68.0
```

The screen lists the protocol name (CUSTOM1 in the example above), protocol step that is running (1), cycle number (1), and the current block temperature (68.0). 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.

Press the right «Select» key to see a screen listing the cycle number, time elapsed, and estimated time remaining in the run:

```
Run: CUSTOM1
Cycle:        1
Total time:    2:20
Est remain: 1:01:51
```

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

Reading the Protocol Completion Screen

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

```
Run: CUSTOM1
  PROGRAM COMPLETE

Total time: 1:20:31
```

Certain error messages may also be displayed in this screen (see Chapter 10). Press «Proceed» to return to the Main Menu.

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: CUSTOM1

    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: CUSTOM1
 1= 92.0  for 0:05
Cycle: 1
Block: 92.0   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: CUSTOM1

Stop CUSTOM1?
        Yes  _NO
```

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

```
Run: CUSTOM 1
     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 PTC-100 cycler 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 PTC-100 cycler 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: CUSTOM 1
    AC POWER FAILED
 Cycle: 1  Step 1
Recovered at 20.2 C
```

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

Using the Instant Incubation Feature

The PTC-100 cycler may be converted to a constant-temperature incubator by pressing «Instant» («0») when the Main Menu is displayed. A screen allowing entry of the incubation temperature will be displayed:

```
INCUBATE:
 1= 37.0  for ever
```

Use the keypad to enter any incubation temperature from 0°C to 100°C, then press «Proceed». The PTC-100 cycler 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».

4 **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

The Elements of a Program

PTC-100® cycler programs consist of a series of steps encoding a protocol. Programs may contain four 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 the block is held at that temperature. The PTC-100 cycler 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 for rates of heating and cooling for all block types.)

2. **Ramp step (optional):** Sets a slower-than-maximum rate of heating or cooling.

3. **Goto step (optional):** Directs the program to cycle back to an earlier step for a specified number of times (up to 9,999 times).

4. **End step (mandatory):** Instructs the PTC-100 cycler 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 Temp:** Modifies a temperature step to allow a "per cycle" increase or decrease of temperature (0.1–10.0°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 annealing temperature begins higher than the calculated temperature, and incrementally decreases each cycle, first reaching, and eventually falling below the calculated annealing temperature. With the reaction beginning at a temperature favoring high stringency in hybridization and incrementing to lower stringency, the reaction favors the desired product by creating a high proportion of signal relative to noise in the early amplification cycles.

2. **Extend Time:** Modifies a temperature step to allow a "per cycle" lengthening or shortening of a temperature step hold (by 1–60 sec/cycle) each time a step is executed. This 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 synthesize during the extension step increases in later cycles because there are more template molecules, because there are fewer active polymerase molecules, or both. The extra time can allow synthesis to be completed.

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 PTC-100 cycler, you may find it helpful to first translate the protocol into PTC-100 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°C for 30 sec
2. 60°C for 3 min
3. 92°C for 30 sec
4. 60°C for 3 min
5. 92°C for 30 sec
6. 60°C for 3 min
7. 92°C for 30 sec

[continues for total of 60 lines]

PTC-100 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

Entering a New Program

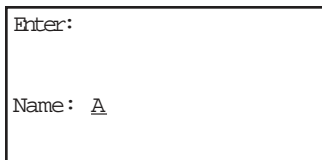Programming the PTC-100 cycler involves four steps:

1. Initiating the program

2. Naming the program

3. Entering the program's steps

4. Entering the end step

Each step involves entering values from the keypad 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 the «.» key returns the machine to Western characters.

When the desired character 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.

Entering the Program's Steps

After the program has been named, the Enter Menu will be displayed:

```
Enter: CUSTOM 1

Step 1= _TEMP Ramp
```

Use this menu to enter the first step of the program:

- *Temp* enters a temperature step.
- *Ramp* enters a ramp 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: CUSTOM 1
 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 keypad to enter any number between 0 and 100.0 as the target temperature (92.0 is used in the example below):

```
Enter: CUSTOM 1
 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: CUSTOM 1
 1= 92.0

Time: _
```

Enter the hold time for the step (30 seconds is used in the example below).

Note: If a hold time of zero (0) is entered, the PTC-100 cycler 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: CUSTOM 1
 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 the "Modifying a Program Step with Options" section in this chapter).

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

Entering a Ramp Step

To enter a ramp step, select Ramp from the Enter Menu. The first ramp screen will be displayed:

```
Enter: CUSTOM 1
 1=


Rate  C / s: _
```

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 ramp rate (in degrees Celsius per second) to be entered for the step.

Use the keypad to enter a slower than maximum ramp rate. Refer to the specifications in Chapter 2 for the maximum rate of heating and cooling for each type of block (0.5°C/sec is used in the example below).

Note: If a ramp rate faster than the block'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: CUSTOM 1
 1= 0.5 /s to


Finish temp:   _
```

Enter the finish temperature for the ramp step (69°C is used in the example below).

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: CUSTOM 1
 1= 0.5 /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 the "Modifying a Program Step with Options" section in this chapter).

4 **Tip:** A ramp step will not hold the block at its finish temperature. Instead, as soon as the 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 (not available as the first program step), select *Goto* from the Enter Menu. The first goto screen will be displayed:

```
Enter: CUSTOM 1
 3=Goto

Goto step: _
```

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 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).

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: CUSTOM 1
 3=Goto,


Addtnl cycles:  _
```

Enter the additional number of times the program should cycle back to the step (29 is used in the example below).

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: CUSTOM 1
 3=Goto 1, 29 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: CUSTOM 1
 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 CUSTOM1 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.

If the folder has been password protected, you will be prompted to enter the password:

```
Save CUSTOM1 in:
<FOLDER1> is secured

Password: _
```

If the correct password is entered, the program will be stored in the folder, and the Main Menu will be displayed.

Modifying a Program Step with 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: CUSTO M 1
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 (see the "Elements of a Program" section at the beginning of this chapter for more information).

- *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 (see the "Elements of a Program" section at the beginning of this chapter for more information).

- *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 of a temperature step, then press «Proceed». The first Inc screen will be displayed:

```
Enter: CUSTOM 1
1= 92.0  for 0:30


C / cycle:  _
```

The temperature step being modified appears on the second line of this screen. The default is 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).

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: CUSTOM 1
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: CUSTOM 1
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 default is to enter a progressive **lengthening** of hold time per cycle. 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).

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: CUSTOM 1
 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: CUSTOM 1
 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 «Cancel» 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: CUSTOM 1
 3= 92.0  for 0:30
 4= 60.0  for 3:00
 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».

To add an option to a step, move the cursor to the step number, then press «Proceed». The Edit Menu will be displayed:

```
Enter: CUSTOM 1
 2=  92.0  for 0:30
    _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: CUSTOM 1

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 the "Revising During Programming" section above.

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 PTC-100 cycler, 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.

7 Editing Programs

Editing a Stored Program

The PTC-100® cycler'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

- Adding and removing temperature and ramp step options.

   Note: The editing tools do not include a renaming function. To rename a program, see the "Renaming a Program" section in Chapter 8.

Initiating Editing

To initiate editing, select *Edit* from the Main Menu, then press «Proceed». One of two screens 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  BOIL
CUT&KILL DENATURE
```

   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).

4   **Tip:** To retain the original version of a program, copy the program (see the "Copying a Program" section in Chapter 8), and then edit the copy.

Editing the Program

The first editing screen displays the first three lines of the program:

```
Edit: CUSTOM 1
1= 92.0  for 0:30
2= 60.0  for 3:00
3=Goto 1, 29 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».

To add or delete a step, or to modify an option, position the cursor on the step number, then press «Proceed». The Edit Menu will be displayed for that step:

```
Edit: CUSTOM 1
 2= 60.0  for 3:00
   _EDIT Insert
Step: Option Delete
```

- *Edit* displays the previous screen again.
- *Insert* allows a step to be added **before** the displayed step.
- *Delete* deletes the displayed step.
- *Option* allows an option to be added to or deleted from 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. The step types available will vary depending on the location where the step will be inserted into the program (a new step 2 is added in the example below):

```
Edit: CUSTOM 1

Step 2= _TEMP Goto
         Ramp End
```

Use the Enter Menu to create the new step (see the "Entering the Program's Steps" section in 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 the "Cancelling Editing Changes" section in this chapter.

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 the "Modifying a Program Step with Options" section in 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.

Deleting an Option

To delete an option modifying a step, select *Option* from the Edit Menu, then press «Proceed». The option menu will be displayed for the step. Select the type of option that you wish to delete from the step and press «Proceed». Select *No* from the confirmation menu that appears at the bottom of the screen.

If deleting a Beep option, the program will be displayed again without the Beep option, and the confirmation menu will reappear. Select *Yes* to accept the change.

If deleting an Inc or Ext option, you will be prompted to enter new values for °C/cycle: _ or sec/cycle: _. Press «Cancel», then select *Yes* from the confirmation menu to delete the option.

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 end step, then press «Proceed». A line allowing the editing session to be ended will be displayed on the last line of the screen:

```
Edit: CUSTOM 1
 4=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: CUSTOM 1

     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

Many of the PTC-100® cycler'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
 ICEBUKET LIGATION
 37°-1HR  37°-6HR
```

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 PTC-100 cycler's memory can hold up to 12 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. A screen asking for the new password will be displayed. Enter the new password. 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.

If the folder is password protected, you will be prompted to enter the password:

```
Delete:
<FOLDER1> is secured.

Password: _
```

After entering the appropriate password, select *Yes* from the confirmation screen, then press «Proceed». The password-protected 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 essentially providing "save as" functionality. Copies can be placed in the original folder or assigned to 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" at the beginning of this chapter), 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>
 <FOLDER3>
```

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" in 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 Files Menu, then press «Proceed». Locate the program to be renamed (see "Locating a Stored Program" at the beginning of this chapter), then press «Proceed». A naming screen will be displayed:

```
Rename:  CUSTOM1



New name: A
```

Name the new program (see the "Naming the Program" section in 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" at the beginning of this chapter), 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" at the beginning of this chapter), then press «Proceed». A confirmation screen will be displayed:

```
Delete:  CUSTOM1

Delete program?
     _YES  No
```

Select *Yes,* then press «Proceed». The program will be deleted, and the Main Menu will be displayed.

If the program is in a password-protected folder, you will be prompted to enter the password. After entering the appropriate password, select *Yes* from the confirmation screen, 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 Utility

Use these utilities, available under List, to view program steps.

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" at the beginning of this chapter), then press «Proceed».

The program will be listed in the LCD window. 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.

Note: Listed programs cannot be edited. To edit programs, select *Edit* from the Main Menu (see Chapter 7).

Setup Utilities

Use these utilities, available from the Setup Menu, to accomplish the following tasks:

- Set a target temperature and a minimum temperature for the Hot Bonnet heated lid
- Look up the PTC-100 cycler's software version number

To display the Setup Menu, select *Setup* from the Main Menu, then press «Proceed»:

```
Setup:
LID      Version


```

Note: The Lid option will only appear if the heated lid is attached.

Choosing a Minimum Block Temperature for the Hot Bonnet Lid

To set a minimum block temperature below which the Hot Bonnet lid will automatically turn off, select *Lid* from the Setup Menu, then press «Proceed». The following screen will be displayed:

```
Lid:
_TARGET   Minimum
```

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

```
Lid Minimum:

  Turn off heated
   lid below 30°C
```

Use the keypad to enter the temperature, then press «Proceed» to enter the value and return to the Main Menu.

Choosing a Target Temperature for the Hot Bonnet Lid

To specify a temperature for the Hot Bonnet lid, select *Lid* from the Setup Menu, then press «Proceed». Select *Target*, and then press «Proceed». The following screen will appear:
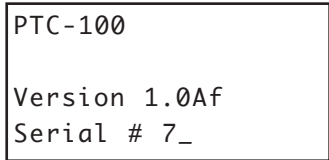
```
Lid target:

105°C
```

Specify a lid temperature at least 10°C higher than any temperature used in the protocol; we suggest 105°C. Press «Proceed» to apply the temperature and return to the Main Menu.

Determining the Software Version Number

At times it is necessary to determine the version number of the software installed in the PTC-100 cycler (e.g., to report a problem to Bio-Rad). To do this, select *Version* from the Setup Menu, then press «Proceed». The software version number and the instrument's serial number will be displayed:

```
PTC-100

Version 1.0Af
Serial # 7_
```

9 Maintenance

Cleaning the Chassis and Block

Clean the outside of the PTC-100® cycler 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% (0.7 M) sodium hypochlorite (household bleach). If using sodium hypochlorite, swab the wells with water afterward to remove all traces of it, or damage to the block's coating may result.

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 (one bleach application at full strength can ruin a block in 3–4 minutes).

Cleaning the Air Vents

Turn off the instrument, and then 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 cycler's heat sink can be hampered, eventually causing overheating and shutdowns (see "Air Supply Requirements" in 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 cleaning methods, monitoring, and disposal of contaminated materials.

Changing the Fuses

The circuits in the PTC-100 cycler are protected by two fuses. When a fuse blows, the PTC-100 cycler 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" in Chapter 5).

⚠ **Warning:**   The PTC-100 cycler 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 cycler. 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. 9-1A). Gently pry the block loose, and pull it down and out. The fuses will be visible (fig. 9-1B).

3. Remove the fuses and examine them. A flat-head screwdriver may be used to pry the fuses loose, if necessary. 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 block back into place, and reconnect the power cord.

Figure 9-1 Changing a fuse.

10 Troubleshooting

Error Messages

Problem	Cause	Action
Instrument Overheated Check Air Flow	Instrument is not getting enough air, or air being taken in is warmer than 31°C.	Correct air supply problems (see "Air Supply Requirements" in Chapter 3) and run protocol again. If error message persists, instrument may need servicing. Call 1-800-4BIORAD (in the US or Canada), or your distributor.
Instrument Overheating Check Air Flow	Instrument is not getting enough air, or air being taken in is warmer than 31°C.	Correct air supply problems (see "Air Supply Requirements" in Chapter 3) and run protocol again. If error message persists, instrument may need servicing. Call 1-800-4BIORAD (in the US or Canada), or your distributor.
AC Power Failed Cycle XX Step X Recovered at XX.X°C	Displayed when a instrument 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 Sensor Fault	Temperature sensor is not working properly.	Instrument needs servicing. Call 1-800-4BIORAD (in the US or Canada), or your distributor.
Lid Sensor Fault	Lid temperature sensor is not working properly. Protocols may still be run, but reactions may fail because of condensation in vessels.	Lid or instrument needs servicing soon. Call 1-800-4BIORAD (in the US or Canada), or your distributor.
HtSink Sensor Fault	The heat sink sensor is not functioning properly.	Instrument needs servicing soon. Call 1-800-4BIORAD (in the US or Canada), or your distributor.
PwrSup Sensor Fault	Power supply sensor is not working properly. Protocols can still be run, but instrument should be serviced soon to repair sensor.	Instrument needs servicing soon. Call 1-800-4BIORAD (in the US or Canada), or your distributor.
Lid Heat Failure	Lid temperature sensor failed during preheat.	Lid or instrument needs servicing. Call 1-800-4BIORAD (in the US or Canada), or your distributor.
Heat Pump Failure	Heat pump sensor is not working properly.	Instrument needs servicing. Call 1-800-4BIORAD (in the US or Canada), or your distributor.

Problem	Cause	Action
No Programs	There are no programs stored in the instrument.	Refer to Chapter 6, Creating Programs" for instructions on creating and entering a new program.
Folder is Empty	There are no programs stored in the folder.	Select a different folder or refer to Chapter 6, Creating Programs" for instructions on creating and entering a new program.
Incorrect password	The password entered does not match the password assigned to the folder.	Enter the correct password for the folder.
Program/Folder name is taken	A program/folder already exists with that name.	Enter a unique program/folder name.
Too many Folders	12 folders already exist.	Either use an existing folder, or first delete a folder and then create a new one.
Secure <Main> Cannot be secured	The Main folder can not be password protected.	If password protection is desired, save the program to a folder other than <Main>.
Memory Low	There is not enough memory left for a maximal size program.	Delete unused programs to make more memory available.
Memory Full	All of the available memory has been filled.	Delete unused programs to make more memory available.
Too Many Programs	The number of stored programs exceeds the maximum allowed.	Delete any unused programs.
Program Too Long	The program size exceeds the maximum allowed.	Reduce the size of the program by using Goto steps and Inc and Ext options (see Chapter 6).

Problems Related to Protocols

Problem	Cause	Action
Reaction is working, but broad low molecular weight band is seen in gels.	"Primer-dimer" material often produces a broad band in the <100 bp 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-complementary.
		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 concentrations of components. May need to reoptimize magnesium concentration.
	Annealing temperature too low.	Reoptimize annealing temperature.
	Protocol contains a wrong value.	Use List to check protocol's 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 to check protocol's temperature 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.
	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 Instrument Performance

Problem	Cause	Action
Beeper sounds continuously.	Hot Bonnet lid has been enabled, but it is not plugged into the instrument.	Cancel protocol. Plug Hot Bonnet lid's cable into its port at back of instrument, and restart protocol.
Block does not heat or cool.	Heat pump is malfunctioning.	Contact Bio-Rad or your distributor.
Instrument will not power up.	Power cable is not plugged into instrument, electrical outlet is dead, or fuse has blown.	Make sure the power cord is plugged into instrument correctly. Make sure electrical outlet is operational. Check fuses.
Instrument starts in middle of a protocol when turned on.	Instrument was turned off before protocol run had finished.	See "Resuming a protocol after a Power Outage" in Chapter 5.
Strange characters appear in LCD window, or text in window freezes or scrolls repetitively.	LCD window or instrument microprocessor is malfunctioning.	Reset the microprocessor by turning instrument off, then back on while pressing the Cancel key at the same time. If problem persists, contact Bio-Rad or your distributor.

Appendix A

Factory-Installed Protocols

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

Appendix B

Warranties

The PTC-100 thermal cycler 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 PTC-100 thermal cycler.

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

For technical service, call your local Bio-Rad office or, in the US, call 1-800-4BIORAD (1-800-424-6723) or visit us on the Web at **discover.bio-rad.com**.

Index

D

E

F

G

H

I

L

M

R

S

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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

The PTC-100® 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 73/23/EEC, 89/336/EEC & 93/68/EEC Directive.

This product is imported into the EU by Bio-Rad Laboratories, Ltd., Bio-Rad House, Maxted Road, Hemel Hempstead (London area), Hertfordshire HP2 7DX England.

13 September, 2004
 date of issue

Brad Crutchfield
Vice President

11144 rev B

Technical Service:

Call your local Bio-Rad office, or in the U.S.
call **1-800-4BIORAD** (1-800-424-6723).

BIO-RAD

**Bio-Rad
Laboratories, Inc.**