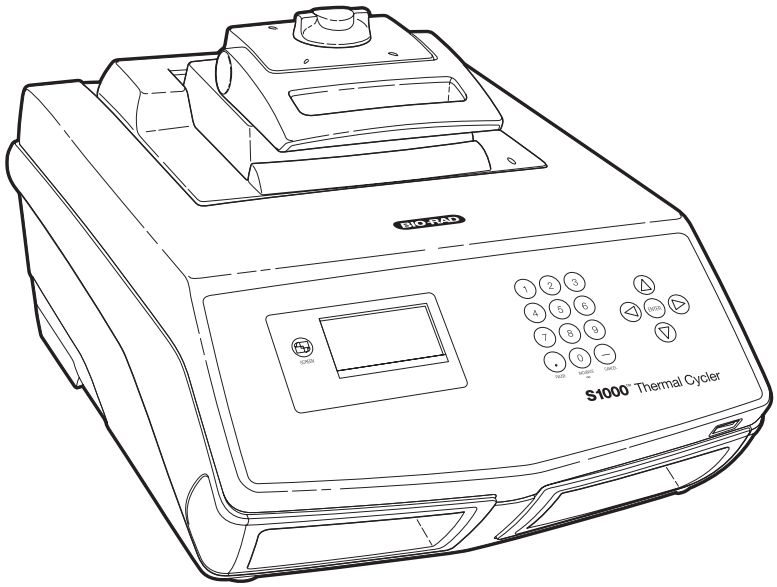
S1000™ Thermal Cycler

Instruction Manual

Catalog # 184-2000
185-2096
185-2048
185-2384



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Bio-Rad Laboratories Resources

Table 1 lists Bio-Rad resources and how to locate what you need.

Table 1. Bio-Rad resources

Resource	How to Contact
Local Bio-Rad Laboratories representatives	Find local information and contacts on the Bio-Rad Laboratories web site by selecting your country on the home page (www.bio-rad.com). Find the nearest international office listed on the back of this manual
Technical notes and literature	Go to the Bio-Rad Laboratories web site (www.bio-rad.com). Type a term in the Search box and select **Literature** to find links to technical notes, manuals, and other literature
Technical specialists	Bio-Rad Laboratories provides quality technical support. We staff our Technical Support department with experienced scientists to provide our customers with practical and expert solutions To find local technical support on the phone, contact your nearest Bio-Rad Laboratories office. For technical support in the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the technical support option

Warranty

The S1000 thermal cycler and associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad Laboratories office for the details of the warranty.

Writing Conventions Used In This Manual

This manual provides instructions on how to safely set up and operate the S1000 thermal cycler and uses the writing conventions shown in Table 2 to quickly provide relevant information.

Table 2. Manual conventions

Convention	Meaning
TIP:	Provides helpful instructions, including information explained in further detail elsewhere in this manual
NOTE:	Provides important information, including information explained in further detail elsewhere in this manual
WARNING!	Explains crucial information about a topic that may lead to injury to the user, instrument damage, or data loss
Screen message	Indicates the one or more words on the screen the user should select

Table 2. Manual conventions (continued)

Convention	Meaning
NAME of control panel key	Indicates a key on the thermal cycler control panel. For example, these keys have the following names: • The **ENTER** key is  • The **right** arrow key is 
Select **X**	Select **X** using the arrow keys. For example, select **NEW** means use the arrow keys to select the **NEW** option on the screen
Select **X > Y**	From menu **X**, select **Y**. For example, select **MAIN > RUN** means select the **RUN** option in the **MAIN** menu
Press **X**	Press the **X** key on the control panel. For example, press **ENTER** means press the **ENTER** key on the control panel

Safety and Regulatory Compliance

The S1000 thermal cycler heats and cools very quickly during operation. We strongly recommend that you follow the safety specifications listed in this section and throughout this manual.

Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Refer to Table 3 to review the meaning of each safety warning label.

Table 3. Instrument safety warning labels

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

Instrument Safety Warnings

The following warning labels display on the instrument, and refer directly to the safe use of this S1000 thermal cycler (Table 4).

Table 4. Instrument safety warning labels

Icon	Meaning
	**Warning about risk of harm to body or equipment**. Operating the S1000 thermal cycler before reading this manual can constitute a personal injury hazard. Only qualified laboratory personnel should operate this instrument
	**Warning about risk of harm to body or equipment from electrical shock.** Do not attempt to repair or remove the outer case of this thermal cycler base, power supply, heat pump, or other accessories. If you open these instruments, you put yourself at risk for electrical shock and void your warranty. All repairs must be done by an authorized repair service
	**Never remove the outer case of a thermal cycler base. This may cause you to receive an electrical shock.** This thermal cycler uses neutral fusing, which means that live power could still be exposed inside the instrument even when the fuse is blown or removed
	**Warning about risk of burning.** A thermal cycler generates enough heat to cause serious burns. Wear safety goggles or other eye protection at all times during operation. Always allow the sample block to return to idle temperature before opening the lid and removing samples. Always allow maximum clearance to avoid accidental skin burns
	**Warning about risk of explosion**. The sample blocks can become hot enough during the course of normal operation to cause liquids to boil and explode

Safety and Regulatory Compliance

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards (Table 5).

Table 5. Safe use specifications

Safe User Requirements		Specifications
Input power	Rated	100–240 Vac, 50–60 Hz
Fuses		250 V, 10 A
Temperature	Indoor use	Ambient temperature of 15–31°C. Relative humidity maximum of 80% (noncondensing)
Altitude		Up to 2,000 meters above sea level
Overvoltage Categories		II
Pollution degree		2

SAFETY COMPLIANCE

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- UL Std No. 61010A-1 Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- UL Std No. 61010A-2-010 Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- CAN/CSA C22.2 No. 1010.1-92 - Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements (includes Amendment 1)
- CAN/CSA C22.2 No. 1010.1B-97 - Amendment 2 CAN/CSA C22.2 No. 1010.1-92 - Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- CAN/CSA C22.2 No. 1010.2.010A-97 - Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials, Amendment No. 1
- IEC 61010-1 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- IEC 61010-1 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use, Part 2: Particular Requirements for Laboratory Equipment for the Heating of Materials

ELECTROMAGNETIC COMPATIBILITY (EMC)

- FCC Title 47 Part 15B as a Class A digital device
- EN61326 Class A Electrical Equipment for measurement, control, and laboratory use - EMC Requirements

FCC WARNINGS AND NOTES

- **Warning.** Changes or modifications to this unit, not expressly approved by the party responsible for compliance, could void the user's authority to operate the equipment
- **Note.** This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference, at his own expense
- **Note regarding FCC compliance.** Although this design of instrument has been tested and found to comply with Part 15, Subpart B of the FCC Rules for a Class A digital device, please note that this compliance is voluntary, for the instrument qualifies as an "exempted device" under 47 CFR 15.103(c), in regard to the cited FCC regulations in effect at the time of manufacture
- **Note regarding Canadian EMC compliance:** Le present appareil numerique n'emet pas de bruits radioelectrique 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
- **Cables.** Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits
- **Use only Bio-Rad USB cable (catalog #184-8000) when using any 1000-series cycler**

Table of Contents

1 Introduction to the S1000™ Thermal Cycler

Read this chapter for information on setting up the S1000 thermal cycler.

System Overview

The S1000 thermal cycler base (Figure 1) includes:

- **Reaction module bay** – holds the inserted reaction module
- **Reaction module locking bar** – locks the inserted module in place
- **Control panel** – provides access to all the functions needed to create and run PCR protocols
- **Air vents** – allow the thermal cycler to heat and cool quickly

Figure 1. Frontal view of the S1000 thermal cycler.

The back panel of the S1000 thermal cycler includes data ports (Figure 2).

- **USB B port** — connects the S1000 thermal cycler to a C1000™ thermal cycler
- **USB A ports** — currently inactive

- **Test port** — for service testing only

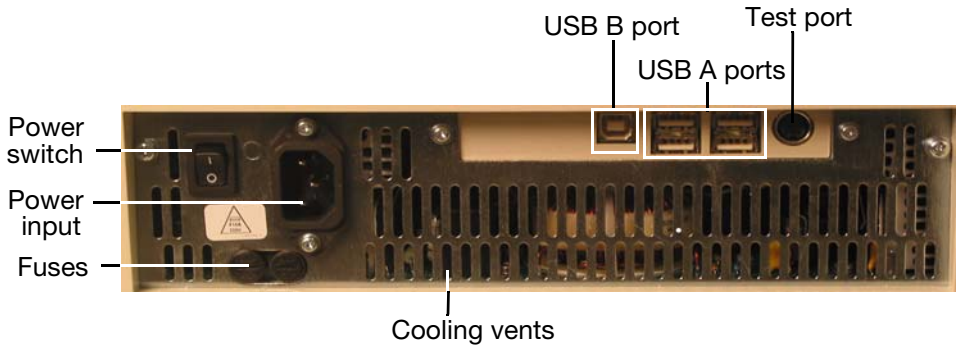

Figure 2. Back panel of the S1000 thermal cycler.

The control panel on the S1000 thermal cycler provides access to all the functions needed to run the thermal cycler and includes the following components:

- **Liquid crystal display (LCD)** — displays the main menu and other screens
- **Command, numeric, and navigation keys** — use these keys to enter commands, numbers, or letters, and navigate various screens

The main screen is displayed after booting is complete. Figure 3 shows the components of the control panel.

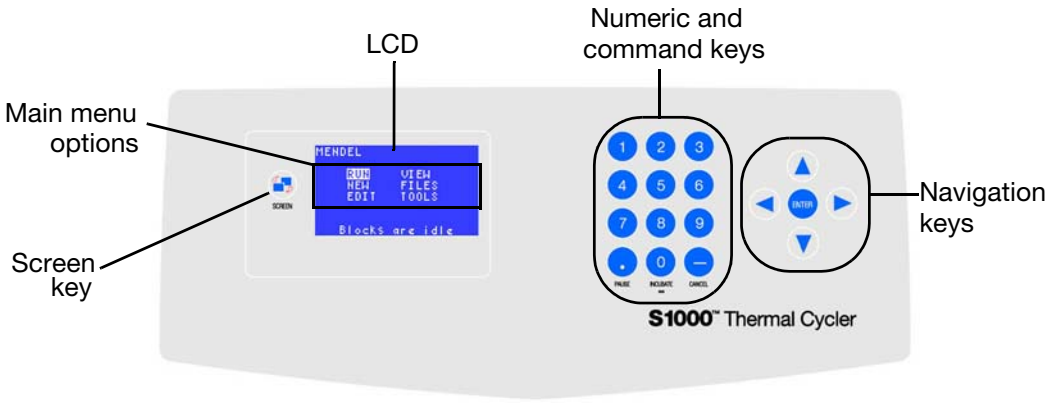

Figure 3. Components of the control panel on the S1000 thermal cycler.

Function of the Control Panel Keys

The control panel of the S1000 thermal cycler contains five sets of keys with the functions listed in Table 6.

Table 6. Function of keys on control panel

Key	Function	Additional Notes
Numeric and Command Keys		
1 through **9**	Enter numbers	
0, **INCUBATE**	Inserts a zero or infinity, or starts instant incubation	

Table 6. Function of keys on control panel (continued)

Key	Function	Additional Notes
CANCEL (**–**)	Enters a minus sign or cancels a function	Press this key to delete an entry, cancel a function, stop a protocol, end an incubation, or delete text on the screen
PAUSE (**.**)	Enters a decimal point or pauses a protocol	
SCREEN key		
	Toggles between screens for alternative views	Press this key to view the status of a run
Navigation Keys		
Right arrow	Moves the cursor to the right	
Left arrow	Moves the cursor to the left	
Up arrow	Moves the cursor up	Press the up arrow key to scroll from **A** to **Z** on the screen. For example, press the up key three times to select the letter **C**
Down arrow	Moves the cursor down	Press the down arrow key to scroll from **Z** to **A** on the screen. For example, press the down key eight times to select the letter **S**
ENTER	Confirms a selection	

Reaction Modules

The S1000 thermal cycler is compatible with any 1000-series reaction module. The reaction modules come in three block sizes: the 96-, dual 48-, or 384-well block. Each block in the reaction module includes a fully adjustable heated lid that is capable of running reliably with a broad range of reaction vessels.

Recommended Sample Volume

When using the S1000 thermal cycler, the maximum sample volume is determined by the type of reaction module used. -well reaction module is used. Table 7 lists the recommended sample volume, as well as the maximum sample volume to be used with different reaction modules.

Table 7. Size and volume limit for the 1000-series reaction modules

Number of Wells	Number of Blocks	Recommended Sample Volume (Upper Limit)
Dual 48/48	2	10–50 µl (50 µl limit)
96	1	10–50 µl (50 µl limit)
384	1	3–30 µl (30 µl limit)

Specifications of Reaction Modules

Specifications for each 1000-series reaction module are listed in Table 8.

Table 8. Reaction module specifications

Feature	96-Well Fast	Dual 48/48 Fast	384-Well
Sample capacity	96 x 0.2 ml tubes	2 x 48 x 0.2 ml tubes	1 x 384-well PCR microplate
Gradient direction	Back (upper temperature) to front (lower temperature) of block	Back (upper temperature) to front (lower temperature) of block	Back (upper temperature) to front (lower temperature) of block
Gradient temperature range	30–100°C	30–100°C	30–100°C
Gradient temperature differential	1–24°C	1–24°C	1–24°C
Gradient accuracy	±0.2°C of programmed temperature at end rows	±0.2°C of programmed temperature at end rows	±0.2°C of programmed temperature at end rows
Gradient (end row) uniformity	±0.4°C well-to-well (within row) within 10 sec of arrival at target temperature	±0.4°C well-to-well (within row) within 10 sec of arrival at target temperature	±0.4°C well-to-well (within row) within 10 sec of arrival at target temperature
Gradient calculator accuracy	±0.4°C of the actual well temperature	±0.4°C of the actual well temperature	±0.4°C of the actual well temperature
Heated lid temperature	0–110°C	0–110°C	0–110°C
Average ramp rate	3.3°C/sec	3.0°C/sec	2.0°C/sec
Maximum ramp rate	5.0°C	4.0°C	2.5°C
Temperature range	0–100°C	0–100°C	0–100°C
Temperature accuracy	±0.2°C of programmed target at 90°C	±0.2°C of programmed target at 90°C	±0.2°C of programmed target at 90°C
Temperature uniformity	±0.4°C well-to-well within 10 sec of arrival at 90°C	±0.4°C well-to-well within 10 sec of arrival at 90°C	±0.4°C well-to-well within 10 sec of arrival at 90°C

Each reaction module contains cooling fins for fast heating and cooling and a fully adjustable, heated lid. Figure 4 shows the lid and cooling fins for the 96-well reaction module.

- **Heated inner lid** — adjusts the lid temperature to prevent condensation and evaporation
- **Sample/reaction block** — holds reaction vessels, including tubes and microplates

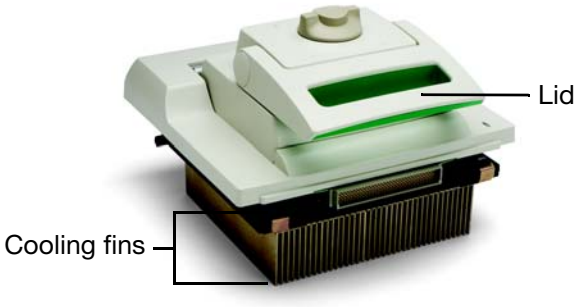

Figure 4. The lid and cooling fins of a 96-well reaction module.

The top of a reaction module lid includes a lid lever, lid force knob, and status LED (Figure 5).

- **Lid lever** — opens and closes the lid
- **Lid force knob** — sets lid force and seals the reaction
- **Status LED** — turns on to indicate that the block is selected or running

Figure 5. A top view of a reaction module.

Setting Up the S1000 Thermal Cycler

The S1000 thermal cycler package includes:

- S1000 thermal cycler base
- Power cord
- Consumables selection guide
- Instruction manual
- Quick guide for system installation

Reaction modules for use with the S1000 thermal cycler are shipped in separate packaging.

Remove all packaging materials and store them for future use. If any item is missing or damaged, contact your local Bio-Rad office.

Place the S1000 thermal cycler base on a flat, dry surface with sufficient cool airflow to run properly. The instrument can run in two modes: stand-alone or software-controlled. When running the system under software-controlled mode, make sure there is sufficient space for a computer during setup.

To insert either a 96-, dual 48-, or 384-well reaction module into the reaction module bay of the thermal cycler base, follow these instructions:

1. With the locking bar in the down position and the lid lever of the reaction module pointing to the front, lift the reaction module into the reaction module bay (Figure 6). Leave about 1–2 cm of space in front of the module.

Figure 6. Inserting the reaction module into the bay.

2. Pull the locking bar up to lock the reaction module in place (Figure 7). There is no space at the front of the module when it is locked into the S1000 thermal cycler base.

   TIP: Store the reaction module in the base when it is not in use.

Figure 7. Locking the reaction module in place.

3. Plug the supplied power cord into the appropriate electrical outlet.
4. Turn on the thermal cycler using the power switch on the back panel of the thermal cycler base.

   NOTE: Before operating the thermal cycler, be sure to read the safety specifications (“Safety and Regulatory Compliance” on page iv) and operating requirements.

5. When the S1000 thermal cycler starts up, it goes through two screens: the black booting and the self-test screens. Once the self-test is run to verify proper functions, the main menu is displayed. Use the main menu to begin operating the thermal cycler.

To remove the reaction module from the thermal cycler base, follow these instructions:

1. **Turn off** the thermal cycler.
2. Unlock and release the reaction module by pushing the locking bar down.
3. Carefully lift the reaction module out of the bay (Figure 8).

   WARNING! Cooling fins may be hot immediately after running a protocol or incubation. Before lifting the reaction module, make sure that the cooling fins are not hot.

4. After removing the reaction module from the S1000 thermal cycler, store it on a clean, flat surface where it cannot get bumped, scraped, or dropped.

   Scraping the cooling fins of the reaction module or dropping the module on the fins could compromise the ability of the module to heat and cool correctly.

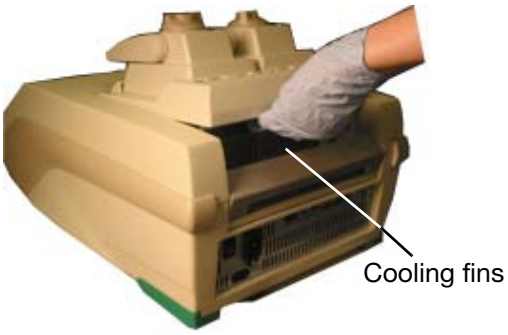

Figure 8. Lifting the reaction module out of the bay.

Operating the Reaction Module Lid

The inner lid of the reaction module applies heat and force to the reaction vessel lids (caps or tape) to produce consistent and successful reactions. Heating the inner lid prevents condensation, while applying force seals the reaction to prevent evaporation.

WARNING! After a run, the heated inner lid can remain hot. Use caution when opening and closing the lid.

To open the lid, use the following steps:

1. Turn the lid force knob counterclockwise to release the inner lid (Figure 9).

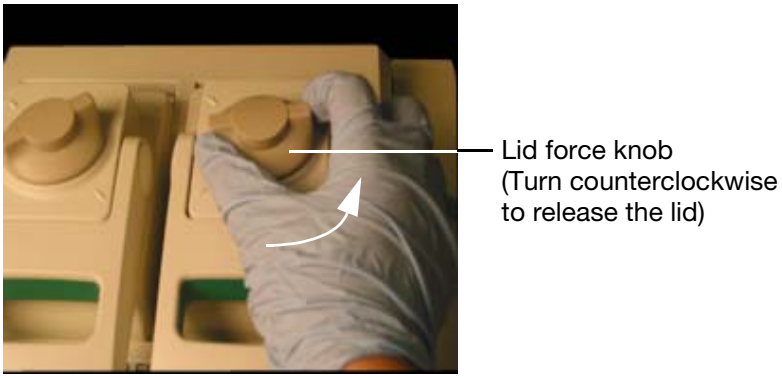

Figure 9. Turn the lid force knob counterclockwise to release the inner lid.

2. To open the lid, push the lid lever back and then lift it up (Figure 10).

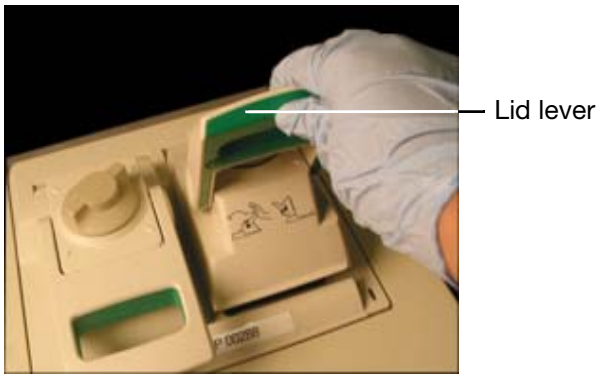

Figure 10. Lift the lid lever up to open the lid.

3. Lift the lid lever completely until the reaction module stays open without assistance.

To close the lid, use the following steps:

1. Push the lid lever down (Figure 11), making sure that the front of the lid is secured beneath the housing, and then lock it in place.

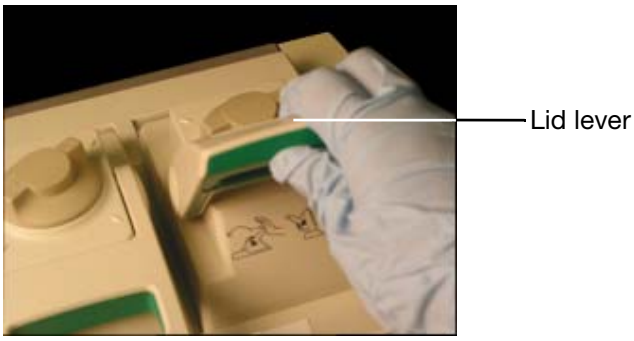

Figure 11. Push the lid lever down.

2. Adjust the lid force by turning the lid force knob (Figure 12).
   - Turn the knob 1/4 clockwise (to the right) to increase the lid force
   - Turn the knob 1/4 counterclockwise (to the left) to decrease the lid force

   Adjust the lid force to a similar setting each time by turning it to the same position.

   NOTE: The position marks on the lid indicate 1/4 turns.

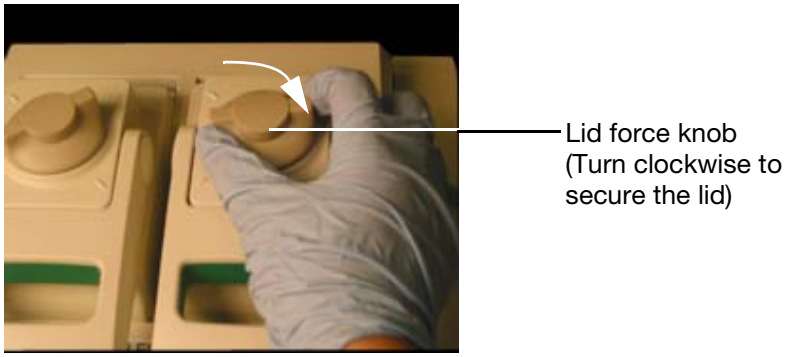

Figure 12. Adjust the lid force by turning the lid lever.

Loading Sample Vessels into the Reaction Block

To ensure uniform heating and cooling of samples, vessels must be in complete contact with the reaction block. Adequate contact is achieved by:

- Confirming that the block is clean before loading samples
- Firmly pressing the individual tubes or the microplate into the block wells

TIP: When using one or a few tubes, make sure to place them in the center of the block to ensure uniform thermal cycling of all samples. Use the tube frame (catalog #184-9000), or load at least one empty tube in each corner of the block to ensure that the lid exerts even pressure on individual tubes.

Main Menu

The main menu (Figure 13) provides access to all thermal cycler operations and displays the status of the reaction module and the name of the thermal cycler.

Figure 13. The main window of the S1000 thermal cycler.

Select the options in the main menu to start these instrument functions:

- **RUN** — to run an existing protocol file
- **NEW** — to create a new protocol file
- **EDIT** — to modify stored protocol files
- **FILES** — to copy, move, rename, delete, or secure protocol files and/or folders
- **VIEW** — to review an existing protocol file
- **TOOLS** — to change thermal cycler settings or to view the last protocol that was run

The File Tree

All protocol files are stored in the file tree. The file tree displays when you need to select a protocol to run, edit, or view.

The file tree (Figure 14) includes these folders:

- **MAIN** folder — stores the preinstalled protocols and cannot be deleted or renamed. The preinstalled files can be run or copied by any user. Do not store user-created protocols in the MAIN folder

- **User folders** — contain user-created protocol files. User folders and associated files can be secured with a password. The files cannot be edited or deleted without using the password

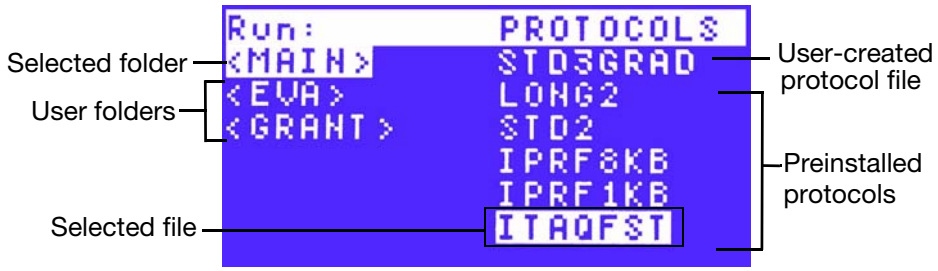

Figure 14. The file tree of the S1000 thermal cycler.

NOTE: If a folder contains more than six protocols, use the arrow keys to scroll down to see all the protocols. All folder names are displayed with angle brackets (**<** and **>**) surrounding the name.

2 Creating and Editing Protocols

Read this chapter for information on creating and editing protocols.

Protocol Steps

Table 9 includes a list of steps in a protocol. The table also includes the limits and range of the parameters.

Table 9. Protocol steps and parameters of the S1000 thermal cycler

Step Name	Parameters and Ranges	Description
TEMP (Temperature)	**Temperature in °C**: The target temperature between 0.0 and 100.0°C in tenths of a degree	Instructs the thermal cycler to ramp to the target temperature, and hold that temperature for the specified amount of time
	Hold time: The hold time between 1 sec and 18 hr in the format of hr:min:sec. To enter an infinite hold, press the ∝ (infinite, **0**) key	

Table 9. Protocol steps and parameters of the S1000 thermal cycler (continued)

Step Name	Parameters and Ranges	Description
GRAD (Gradient range)	**Lower**: The lower temperature in the gradient. Enter a number between 30.0 and 99.0°C in tenths of a degree **Upper**: The upper temperature in the gradient. The maximum temperature is 100°C. Enter a temperature within 24.0°C of the lower temperature	Instructs the thermal cycler to ramp to the target temperature gradient across the block, and hold that temperature gradient for the specified amount of time
	Time:The hold time between 1 sec and 18 hr in the format of hr:min:sec. To enter an infinite hold, press the ∝ key (infinite, **0**) key	
GOTO	**GOTO step**: The step number of the first step in the repeat.	A protocol step that instructs the thermal cycler to repeat a set of steps for the specified number of times. NOTE: The total number of cycles in the protocol is the number of **GOTO** repeats, plus the first cycle.
	ADDTNL REPEATS: The number of additional times that the steps repeat.	
END	(No parameters)	A protocol step that instructs the thermal cycler to finish the protocol

Creating a New Protocol

NOTE: The internal memory of the S1000 thermal cycler can hold up to 400, 2-step protocols.

To create a protocol:

1. Select **NEW** from the main menu (Figure 15). Press **ENTER** to confirm the selection.

Figure 15. Select NEW from the main menu.

2. Use the numeric keys to enter the name of the new protocol file. Enter a letter by pressing the up or down arrow key and a number by pressing the numbered key. For example, to select the letter **C**, press the up key 3 times. To select the letter **S**, press the

down key eight times. Press **ENTER** to continue to the next space. Press **ENTER** to continue to the next screen.

NOTE: A protocol file name can contain 1–8 characters and must be unique to the folder. To delete or change a letter, press **CANCEL** and select a new letter. To delete the entire name, press **CANCEL** multiple times.

In Figure 16, the characters **STD3** are entered, and the cursor is highlighting the next space.

Figure 16. STD3 is entered as the protocol name.

3. (Optional) Enter a new lid temperature, and press **ENTER** to continue to the next screen.

   NOTE: The lid temperature can range from 0 to 110°C. When the block is running an infinite hold at a temperature below the **Turn off below** parameter, the lid heater maintains 31.0°C. To change the default **Turn off below** parameter, select **TOOLS** > **DEFAULTS**.

4. (Optional) Enter the sample volume in microliters (µl), and press **ENTER** to continue to the next screen.

   NOTE: Entering a sample volume between 1 and 50 selects **Calculated Temperature** control mode, which is the standard mode. Entering **zero** (**0**) in the volume field selects **Block** mode. Calculated mode is the recommended mode because it most accurately represents the actual sample temperature. For more information about Temperature control modes, see page 27.

5. Using the arrow keys, select **TEMP** to enter a temperature step or **GRAD** to enter a gradient temperature step in the protocol file. Press **ENTER** to continue to the next screen.

   In Figure 17, **TEMP** is selected as the temperature step.

Figure 17. TEMP is selected as the temperature step in this protocol file.

NOTE: The first step in a protocol must be either a **TEMP** or **GRAD** step.

6. Enter the target temperature between 0 (zero) and 100.0°C for the temperature step. Press **ENTER** to continue to enter the next item in the protocol.

In Figure 18, the target temperature is **95°C.**

Figure 18. Target temperature of 95°C is used in this protocol.

7. Enter the hold time (**TIME**) in minutes and seconds using the numeric keys. The hold time (**TIME**) ranges between 0:01 (one second) and 18:00:00 (18 hours). Entering 0 (zero) adds an infinite hold and holds this step **FOREVER**. Press **ENTER** to continue to the next field.

   For example, to enter 4 minutes (4:00), type **400**. To enter 30 seconds, type **30**. In Figure 19, the hold time is four minutes.

Figure 19. The hold time is 4 minutes in this protocol.

8. Select **YES**, **No**, or **Option** by pressing the right and left arrow keys. Press **ENTER** to continue to the next screen.
   - **YES** — to confirm the current parameters for this protocol step
   - **No** — to change a parameter in this protocol step
   - **Option** — to add more parameters to this protocol step. For more information about entering options, see “Adding an Increment to a Temperature Step” (page 19)

9. (Optional) Enter a gradient temperature step by pressing the right arrow key to select **GRAD** (Figure 20). Press **ENTER** to continue to the next screen.

   NOTE: A temperature gradient is limited to a 24°C spread. The lowest possible “lower” temperature in the gradient is 30°C and the highest “upper” temperature is

100°C. Therefore the lowest gradient is **30–54°C**, and the highest possible gradient is **76–100°C**.

Figure 20. GRAD is selected in the gradient temperature step.

TIP: Check the temperature in each row of the block in a gradient by selecting the gradient calculator tool (**TOOLS** > **GRADCALC** on page 58).

10. Enter the lower temperature in the gradient. The lower temperature is at the front (row H) of the block.

    In Figure 21, the lower temperature is 50°C.

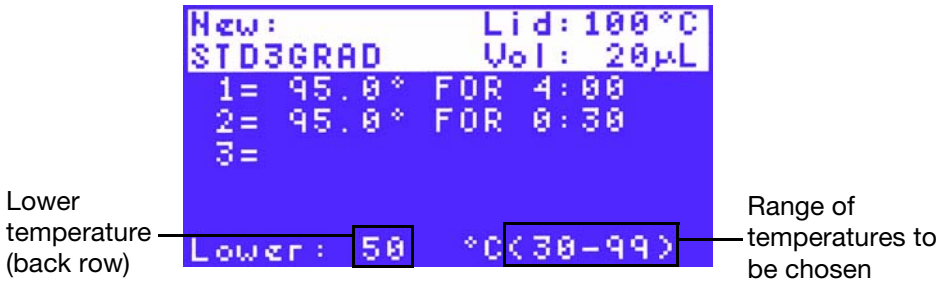

Figure 21. The lower temperature is 50°C in this protocol.

11. Enter the upper temperature in the gradient. The upper temperature is at the back (row A) of the block.

    NOTE: The range of temperature is limited by the widest available range for gradient, which is 24°C. The **highest** value that can be entered for the upper temperature is 100°C.

12. Enter a hold time between 0:01 (one second) and 18:00:00 (18 hours).

13. Select **YES**, **No**, or **Option** by pressing the right and left arrow keys, then press **ENTER** to continue to the next screen:
    - **YES** — to confirm the current parameters for this protocol step
    - **No** — to change a parameter in this protocol step
    - **Option** — to preview the temperature gradient. If **Option** is selected, select **VIEW** on the next screen to view the gradient or **EXT** to add a hold time extension. Press **ENTER** again to return to the previous screen

In Figure 22, the gradient is formed on a 96-well block with a range from **55–75°C**. This screen displays the approximate temperature of each row of the block, and labels the front and back rows.

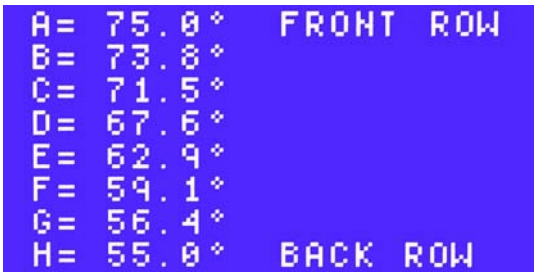

Figure 22. The approximate temperature of each row of the block.

14. Repeat the instructions in steps 6–9 to continue entering additional temperature steps. In Figure 23, four steps are entered.

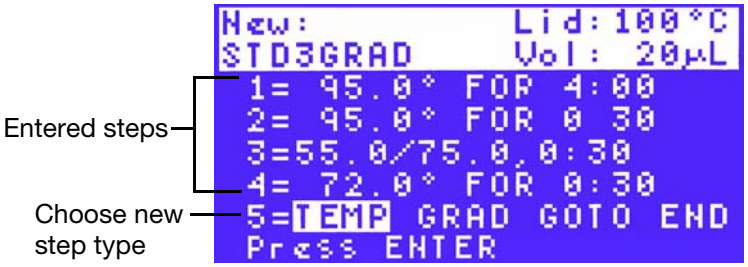

Figure 23. Enter all the temperature steps.

NOTE: A protocol can contain up to 99 protocol steps. The first step must be a temperature (**TEMP**) step, while the last step must be an **END** step.

15. (Optional) To enter a **GOTO** step immediately after the set of steps to be repeated in a cycle, use the arrow keys and select **GOTO**. Press **ENTER** to continue to the next screen.

    For more information about how the **GOTO** step creates a cycle, see “Protocol Steps” (page 11).

    In Figure 24, step 5 is a GOTO step.

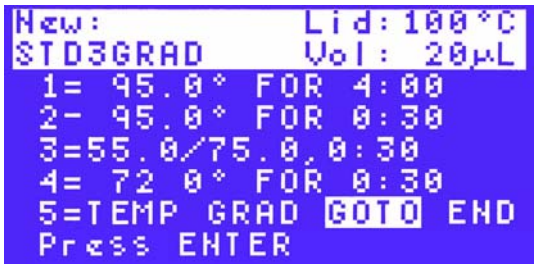

Figure 24. A GOTO step is selected.

NOTE: The **GOTO** step cannot be the first or the last step in the protocol.

16. Enter the step number for the first step in the **GOTO** repeats using the numeric keys. Press **ENTER** to continue to the next screen.

In Figure 25, the first step is **2.** The **GOTO** step instructs the thermal cycler to return to step 2 and repeat all the steps between steps 2 and 5.

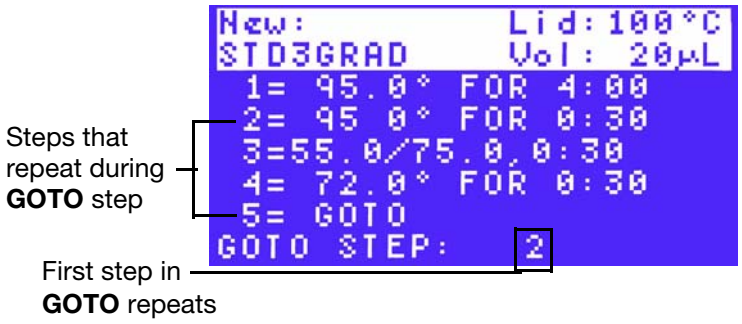

Figure 25. Enter the first step in the GOTO repeats.

17. Enter the number between 1 and 9999 for the additional repeats (**ADDTNL REPEATS**) in the **GOTO** step. Then press **ENTER** to continue to the next screen.

    NOTE: The **GOTO** step adds additional cycles to the PCR protocol. The first cycle is not included in the **GOTO** step. For example, to run a PCR protocol with 31 cycles, enter 30 repeats in the **GOTO** step.

    In Figure 26, the number of repeats is **30**, and the total number of cycles is **31.**

Figure 26. Enter the number of repeats in a GOTO step.

18. Select **YES** to accept the **GOTO** step parameters (Figure 27), or select **No** to return to the beginning of this step and change the **GOTO** step parameters. Then press **ENTER** to continue to the next screen.

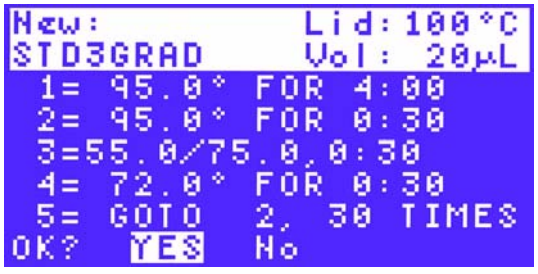

Figure 27. Confirm a GOTO step by selecting YES.

19. Enter the remaining steps by choosing the step type and adding parameters. Then press **ENTER** to continue to the next screen.

    TIP: To instruct the thermal cycler to emit a sound at the end of the protocol, include a **BEEP** option in the final temperature step (page 21).

20. Select **END** using the arrow keys to instruct the thermal cycler to finish the protocol file. Press **ENTER** to continue to the next screen. In Figure 28, the **END** step is selected.

    NOTE: The **END** step must be the last step of a protocol; a protocol can only contain one **END** step.

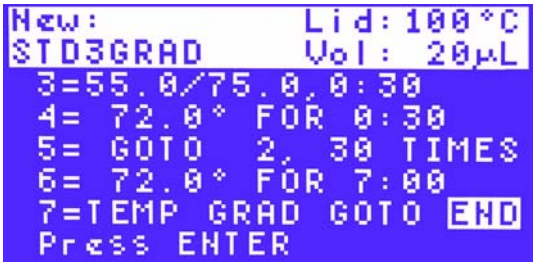

Figure 28. The END step in a protocol.

21. Select **YES** (Figure 29) to accept the protocol step parameters or **No** to return to the beginning and select a different protocol step.

Figure 29. Select YES to accept the protocol parameters.

22. Use the arrow keys to select the folder where you want the new protocol file to be saved, and press **ENTER** to save the protocol file.

    NOTE: The file tree folder screen does not appear if there are no user-created folders (i.e. the MAIN folder is the only folder on the list). Saving the protocol in the **MAIN** folder is not recommended. If the protocol is saved in the MAIN folder, we recommend moving it to a user-created folder. (See “Moving a Protocol File” on page 41 for more information.)

    In Figure 30, the **STD3GRAD** file is saved in the **GRANT** folder.

Figure 30. The STD3GRAD file is to be saved in the GRANT folder.

To run a protocol, follow the instructions in “Preparing to Run a Protocol” on page 29.

Parameters for Temperature or Gradient Steps

Table 10 includes a list of options for temperature and gradient steps for the S1000 thermal cycler. The table also includes the limits and range of the parameters.

Table 10. Options and parameters for protocols on the S1000 thermal cycler

Step Name	Parameters and Ranges	Description
INC (Increment)	A temperature from –10.0 to 10.0°C per cycle in tenths of a degree	Applies only to a temperature step (see "Adding an Increment to a Temperature Step" on page 19). Instructs the thermal cycler to increment (change) the target temperature of a step with each cycle, where a positive number increases the temperature and a negative number decreases the temperature
EXT (Extend)	A time from –60 to 60 sec per cycle	Applies to both temperature and gradient steps (see "Extending the Hold Time in a Temperature Step" on page 20). Instructs the thermal cycler to extend the hold time with each cycle. A positive number increases the hold time and a negative number decreases the hold time
RATE (Ramp rate)	A number from 0.1 to 5°C per sec	Applies only to a temperature step (see "Changing the Ramp Rate in a Temperature Step" on page 21). Instructs the thermal cycler to ramp to the target temperature at the specified ramp rate in that step
Beep	(No parameters)	Applies only to a temperature step (see "Adding a Beep to a Temperature Step" on page 21). Instructs the thermal cycler to beep to signal that the thermal cycler has reached the target temperature for that step

Adding an Increment to a Temperature Step

The increment (**INC**) parameter changes the target temperature of a protocol step. The increment can increase or decrease the target temperature with each cycle in the protocol.

To add an increment:

1. Select **OPTION** using the arrow keys. Press **ENTER** to continue to the next screen.

In Figure 31, **OPTION** is selected to add an increment to a temperature step.

Figure 31. Select OPTION to add an increment to a temperature step.

NOTE: The **INC** parameter must be added to a step within the **GOTO** repeats in order to increment with each cycle of the reaction.

2. Select **INC** using the arrow keys (Figure 32) to add an increment to the protocol step in each cycle. Press **ENTER** to continue to the next screen.

Figure 32. Select INC to add an increment.

3. Enter the increment temperature using the numeric keys. To decrease the temperature each cycle, enter a negative number by pressing the **CANCEL** (–) key. Press the **PAUSE** (**.**) key to enter a decimal point. Then press **ENTER** to continue to the next screen.

   NOTE: Enter an increment from –10.0 to 10.0°C per cycle in tenths of a degree, and within the limits of a temperature step (0–100°C).

   In Figure 33, the increment is +0.5°C. The target temperature for step 3 will increase by 0.5°C each cycle.

Figure 33. Enter the increment temperature.

4. To confirm the parameters of the protocol step, select **YES** and then press **ENTER**. To change the parameters, select **No** and then press **ENTER**.

Extending the Hold Time in a Temperature Step

The **EXT** parameter changes the hold time for a temperature or gradient temperature step. The extension increases or decreases the hold time with every cycle.

To add an extension:

1. Select **OPTION** using the arrow keys. Press **ENTER** to continue to the next screen.
2. Select **EXT** using the arrow keys to add an increment to the protocol step in each cycle. Press **ENTER** to continue to the next screen.

   NOTE: The **EXT** option must be added to a step within a **GOTO** repeat in order to extend with each cycle of the reaction.
3. Enter the extension time in seconds using the numeric keys. To decrease the hold time in each cycle, enter a negative number by pressing **CANCEL** (–). Then press **ENTER** to continue to the next screen.

   NOTE: Enter an extension time from –60 to 60 seconds per cycle in whole numbers. The time entered must be between tenths of a degree. This value should also be within the limits of a temperature step, which is 1 second to 18 hours.
4. To confirm the parameters of the protocol step, select **YES** and then press **ENTER**. To change the parameters, select **No** and then press **ENTER**.

Changing the Ramp Rate in a Temperature Step

The **RATE** parameter changes the ramp rate of a temperature step. The ramp rate is the rate at which the thermal cycler heats or cools to the target temperature of a step.

To change the rate, follow these instructions:

1. Select **OPTION** after entering the initial temperature step parameters, and then press **ENTER** to continue to the next screen.
2. Select **RATE** to change the ramp rate (Figure 34). Press **ENTER** to continue to the next screen.

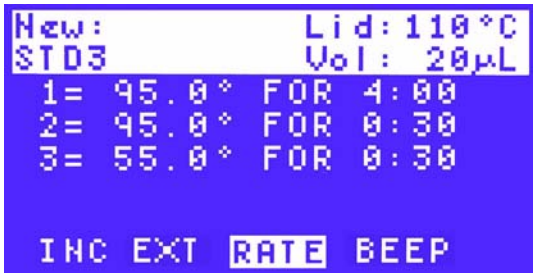

Figure 34. Select RATE to change the ramp rate.

3. Enter a ramp rate (in °C/sec) using the numeric keys. Press **PAUSE** (**.**) to enter a decimal point.

   NOTE: Enter a ramp rate between 0.1 and 5°C/sec in tenths of a degree.
4. To confirm the parameters of the protocol step, select **YES** and then press **ENTER**. To change the parameters, select **No** and then press **ENTER**.

Adding a Beep to a Temperature Step

The **BEEP** parameter instructs the thermal cycler to emit a sound when the temperature reaches its target. A **BEEP** can be added to any temperature step.

TIP: Add the beep step to a temperature step, such as an infinite (**FOREVER**) hold, to have the thermal cycler give a signal when it initiates the step.

To add a beep:

1. Select **OPTION** after entering the initial temperature step parameters, and then press **ENTER** to continue to the next screen.
2. Select **BEEP** to signal the end of the protocol step. Press **ENTER** to continue to the next screen.

   NOTE: The **BEEP** parameter can only be added to a temperature step.
3. To confirm the parameters of the protocol step, select **YES** and then press **ENTER**. To change the parameters, select **No** and then press **ENTER**.

Editing an Existing Protocol

NOTE: A protocol that is already running cannot be edited. Changes made in a protocol that is running apply to the next time the protocol runs. To stop editing a protocol, press **CANCEL** several times.

Editing the Lid Temperature and Sample Volume

To edit an existing protocol:

1. Select **EDIT** from the main menu (Figure 35). Press **ENTER** to confirm the selection.

Figure 35. Select EDIT from the main menu.

2. Using the arrow keys, select the folder that contains the protocol file to be edited. Press **ENTER** to continue to the next screen.

   In Figure 36, the file named **STD3** is selected in the folder named **EVA**.

Figure 36. THE STD3 file in the EVA folder is selected.

3. Enter the new lid temperature (optional) or use the default lid temperature. Press **ENTER** to accept the lid temperature and continue to the next screen.

   NOTE: The lid temperature can range from 0 to 110°C. When the block is running an infinite hold at a temperature below the **Turn off below** parameter, the lid heater maintains 31.0°C. To change the default **Turn off below** parameter, select **TOOLS** > **DEFAULTS**.

4. Enter a new sample volume (optional) or use the default volume. Press **ENTER** to continue to the next screen.

   NOTE: Entering a sample volume between 1 and 50 selects **Calculated Temperature** control mode, which is the standard mode. Entering **zero (0)** in the volume field selects **Block** mode. Calculated mode is the recommended mode because it most accurately represents the actual sample temperature. For more information about temperature control modes, see page 27.

Inserting a Protocol Step

1. Select **EDIT** from the main menu (Figure 37). Press **ENTER** to confirm the selection.

Figure 37. Select EDIT from the main menu.

2. Using the arrow keys, select the folder that contains the protocol file to be edited. Press **ENTER** to continue to the next screen.

3. Select a protocol step to edit using the arrow keys. Press **ENTER** to continue editing the step.

   In Figure 38, step **4** is selected for editing.

Figure 38. Select the protocol step to be edited.

4. Select **INS** to insert a step above the selected protocol step. In Figure 39, **INS** is selected to insert a step above step **4**.

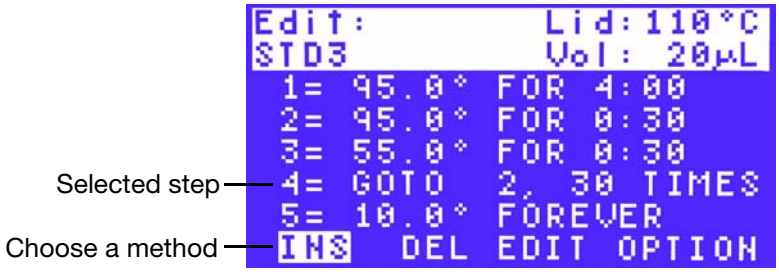

Figure 39. INS is selected to insert a step above step 4.

5. Select **TEMP**, **GRAD**, or **GOTO** as the type of protocol step to be inserted. Press **ENTER** to continue to the next screen.

In Figure 40, a temperature step (**TEMP**) is selected.

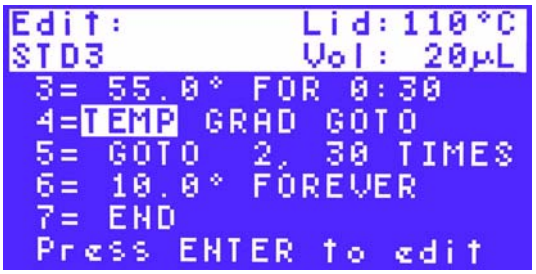

Figure 40. TEMP is selected as the type of protocol step to be inserted.

6. Enter the step parameters, then press **ENTER** to confirm each parameter.

   In Figure 41, the target temperature of 72°C is entered.

Figure 41. The temperature of the inserted step is entered.

7. (Optional) Enter more step parameters by selecting **OPTION**. For example, add an increment or extension to this temperature step. For more instructions about entering additional parameters to a step, see “Parameters for Temperature or Gradient Steps” on page 19.

   In Figure 42, **OPTION** is selected to add parameters to step 4.

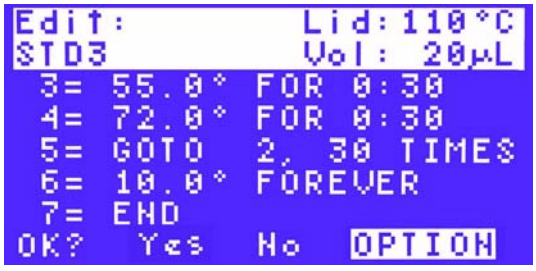

Figure 42. Select OPTION to add additional parameters.

8. Enter the parameters of the new step. Then press **ENTER** to confirm each parameter.

Deleting a Protocol Step

1. Select **EDIT** from the main menu. Press **ENTER** to confirm the selection.
2. Using the arrow keys, select the folder that contains the protocol file to be edited. Press **ENTER** to continue to the next screen.
3. Select a protocol step to delete using the arrow keys. Press **ENTER** to continue editing the step.
4. Select **DEL** to delete the selected protocol step. In Figure 43, step **4** is selected to delete. Press **ENTER** to continue to the next screen.

In Figure 43, step **4** is selected to be deleted.

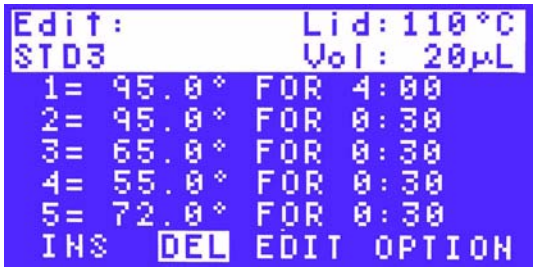

Figure 43. Select DEL to delete a protocol step.

5. Delete the selected step. Press **ENTER** to delete the step and continue to the next screen. Notice that the deleted step parameters are replaced with the parameters of the next step.
6. Confirm the deletion. When prompted with **Save changes?**, select **YES** and press **ENTER** to delete the step. Alternatively, select **No** and press **ENTER** to return to the beginning of this step.

   In Figure 44, **YES** is selected.

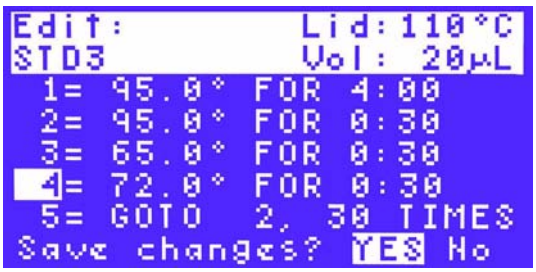

Figure 44. Select YES to delete the selected step.

Editing a Protocol Step

To change the parameters in the existing protocol steps, use the following instructions:

1. Select **EDIT** from the main menu. Press **ENTER** to confirm the selection.
2. Using the arrow keys, select the folder that contains the protocol file to be edited. Press **ENTER** to continue to the next screen.
3. Select a protocol step to delete using the arrow keys. Press **ENTER** to continue editing the step.
4. Select **EDIT** to delete the selected protocol step. Press **ENTER** to continue to the next screen.

   TIP: When you first select **EDIT,** you can edit the parameters in the selected protocol step. To edit the parameters in a different protocol step, press the arrow keys to go to that step.

In Figure 45, step **4** is selected to be deleted. Notice the original parameter for temperature is 55°C.

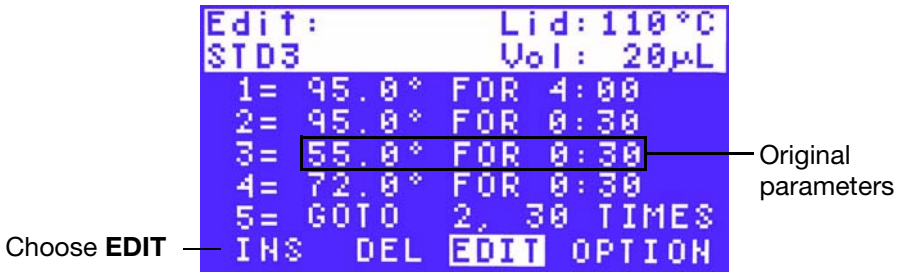

Figure 45. Select EDIT to delete a protocol step.

5. Change the first parameter in the step by pressing the keys on the control panel to enter the temperature. Then press **ENTER** to continue to the next parameter.

   In Figure 46, the temperature is changed from 55 to 65°C.

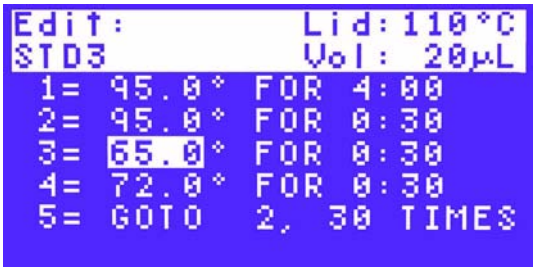

Figure 46. Enter a new temperature.

6. Change the second parameter in the step by pressing the numeric keys to type a new parameter. Then press **ENTER** to continue to continue to the next step.

   In Figure 47, the hold time is changed to **35** seconds.

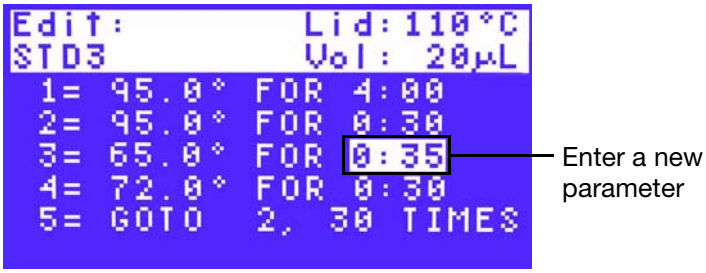

Figure 47. Enter a new hold time.

7. Continue editing the existing parameters in each step (optional). Press the up and down arrow keys to choose an earlier or later step in the protocol.

In Figure 48, the temperature parameter is changed to 70.0°C in step 4.

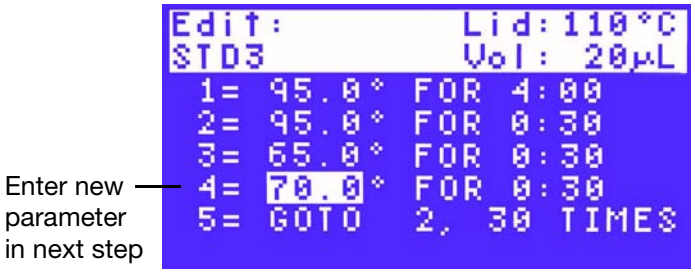

Figure 48. The temperature parameter is changed from 72.0°C to 70.0°C.

8. Press **ENTER** to finish changing parameters in the step and to continue editing the protocol.

Sample Volume and Lid Temperature

The sample volume and lid temperature influence the outcome of a PCR protocol.

- **Sample volume** — determines the temperature control mode, which influences the amount of time the samples are held at the target temperature
- **Lid temperature** settings — determine the temperature of the heated lid and when it cuts off. If the temperature is too high, the sample temperature might rise above the target temperature

The S1000 thermal cycler provides various ways of managing and entering sample volume and lid temperature settings:

- Change the setting when creating a protocol (page 12)
- Change the settings when editing the protocol (page 22)
- Change the settings when preparing to run a protocol (page 29)

Temperature Control Modes

The S1000 thermal cycler uses one of two temperature control modes to determine when the sample reaches the target temperature:

- **Calculated mode** — the thermal cycler calculates the sample temperature based on the sample volume when a sample volume between 1 and 50 µl is entered for 96- or dual 48-well reaction modules or a volume between 1 and 30 µl is entered for the 384-well reaction module. The calculated mode is recommended, because it most accurately represents the actual sample temperature
- **Block mode** — when a sample volume of zero (0) µl is entered, the thermal cycler assumes that the sample temperature is the same as the measured block temperature

Choosing the Appropriate Lid Temperature

The adjustable heated lid of the reaction module allows the user to control the lid temperature and force. Heating the lid prevents condensation from forming inside the tubes and plates.

When the S1000 thermal cycler is running, the heated lid maintains the temperature specified for the protocol being run. Without a heated lid, water can be lost from the reagents to condensation, concentrating the reactants in the tube or plate.

The default lid temperature of the S1000 thermal cycler is 105°C for 96- or dual 48-well reaction blocks and 95°C for 384-well blocks.

NOTE: When the block is running an infinite hold at a temperature below the **Turn off below** parameter, the lid heater maintains 31.0°C. The default **Turn off below** setting is 30.0°C. To change the default **Turn off below**, select **Tools** > **Defaults**.

3 Running Protocols

Read this chapter for information on setting up the S1000 thermal cycler.

Preparing to Run a Protocol

NOTE: You can run a protocol in a secure folder without entering the password first. See “Securing Files in a Folder” on page 43 for more information about files in secure folders.

To run a protocol:

1. Load the samples in the block. Close the lid and set the lid force using instructions on page 8.
2. Select **RUN** from the main menu. Press **ENTER** to confirm the selection and continue to the next screen.

   NOTE: The main menu status should show **Block is idle** (Figure 49). With a dual 48/48 reaction module, the status message is **Block are idle** when the blocks are both available to run a protocol.

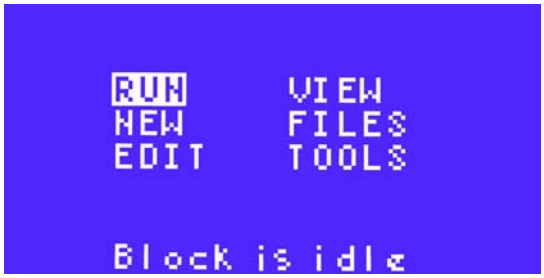

Figure 49. Select RUN from the main menu.

3. Select a folder that contains the protocol file of interest, and then press the right arrow key to select the file. Press **ENTER** to confirm the selection and continue to the next screen.

   NOTE: Select a protocol from the preinstalled protocols from the **MAIN** folder or any user folder in the file tree.

   In Figure 50, the preinstalled protocol **ITAQFAST** is selected in the **MAIN** folder.

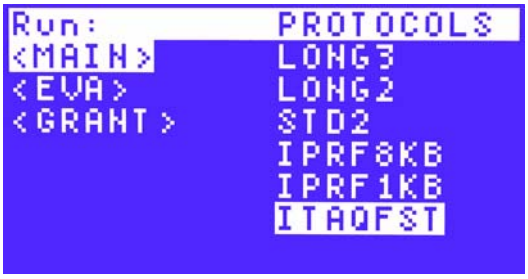

Figure 50. Select the folder and protocol file.

4. Select **BLOCK A** or **BLOCK B** using the right and left arrow keys. Press **ENTER** to continue to the next screen. In Figure 51, **BLOCK A** is selected

   NOTE: When a block is selected, the LED on the reaction module lights up. In a dual 48/48 reaction module, **BLOCK A** appears on the left side.

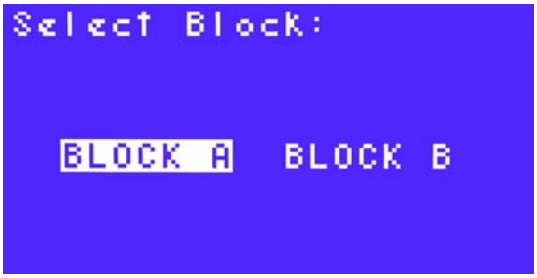

Figure 51. Block A is selected.

5. Enter the sample volume. To use the Calculated mode (standard), enter a value between 1 and 50 µl. To use Block mode, enter 0 (zero).

   NOTE: Calculated mode is the recommended temperature control mode because it most accurately represents the actual sample temperature. See “Temperature Control Modes” on page 27.

6. Press the numeric keys to enter a new sample volume, then press **ENTER** to continue to the next screen.

In Figure 52, the sample volume is 25 µl.

Figure 52. Enter a new sample volume.

NOTE: When running a dual block, the message is **RUN ITAQFST on A**, where **ITAQFST** is the protocol and **A** is the block.

7. (Optional) Select **VIEW** to review the protocol before starting the run (Figure 53), and press **ENTER** to confirm the selection.

Figure 53. Select VIEW to review the protocol.

NOTE: While reviewing the protocol, press **ENTER** to scroll down through the steps in the protocol. When you reach the last step in the protocol, press **ENTER** again to exit. In Figure 54, the screen shows the steps in the **ITAQFST** protocol.

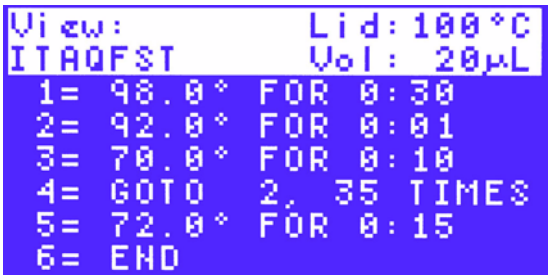

Figure 54. View the steps of the protocol.

8. Select **RUN** using the arrow keys (Figure 55), and press **ENTER** to start running the protocol.

Figure 55. Select RUN to start running the protocol

Monitoring the Protocol Run

Once the run begins, the progress of the run can be monitored with any one of the following screens:

- **Running screen** – displays the current step parameters, temperature, hold time, and cycle (Figure 56)
- **Graphical screen** – displays a graph that approximates the relationship of the target temperatures in each step in the protocol. Each step is listed by temperature only (Figure 57)
- **Time Remaining screen** – shows the amount of time remaining until the end of the protocol (Figure 58)

To monitor the run:

1. Press **SCREEN** on the control panel.

   The **Running** screen appears as a default (Figure 56). If **SCREEN** is pressed again, the instrument shows **graphical** screen (Figure 57). Press **SCREEN** again to see the **Time Remaining** screen (Figure 58).

   NOTE: For the dual 48/48 reaction module, the thermal cycler displays these screens for **Block A** and then for **Block A**. Pressing **SCREEN** toggles the display through each screen and both blocks.

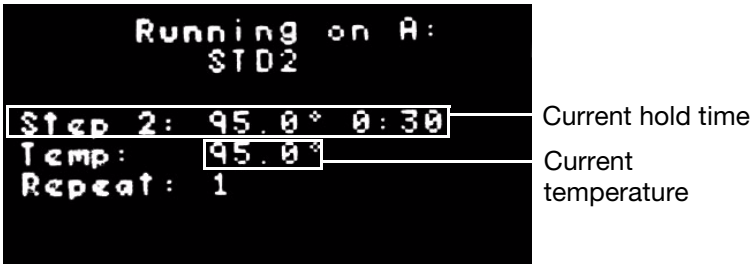

Figure 56. The Running screen is displayed.

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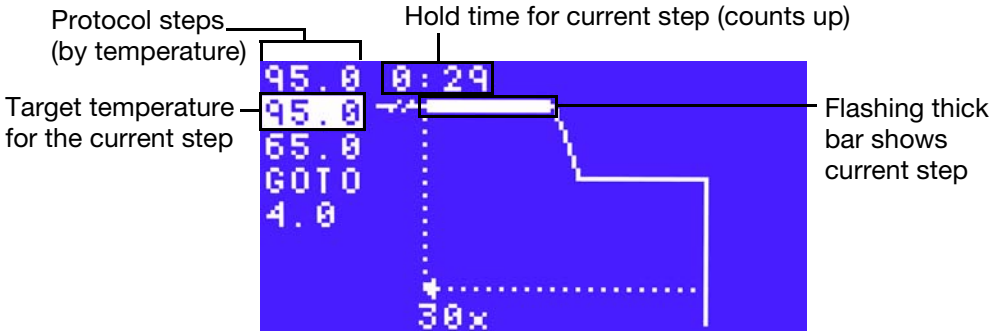

Figure 57. The graphical screen is displayed.

Figure 58. The Time Remaining screen.

2. When the S1000 thermal cycler completes running the protocol, the **PROTOCOL COMPLETE** screen is displayed (Figure 59). After viewing this screen, press **ENTER** to return to the main menu.

Figure 59. PROTOCOL COMPLETE screen is displayed with the run is completed.

3. (Optional) Press **SCREEN** to see a synopsis of the last protocol that was run. The protocol summary is displayed on the **LAST RUN** screen (Figure 60).

   NOTE: For dual 48/48 blocks, press **SCREEN** again to view the **LAST RUN** screen for each block.

Figure 60. Summary of the last protocol is listed on the LAST RUN screen.

   TIP: The **LAST RUN** screen is also available in the **TOOLS** option (page 53).

4. Press **SCREEN** again to return to the main menu

Pausing and Resuming a Run

A running protocol may be temporarily paused. During a pause, the thermal cycler maintains the block temperature at the current target temperature. If the block temperature has not reached the target temperature, then the thermal cycler continues to heat or cool until it reaches that target temperature.

WARNING! Pausing a protocol can alter the results of your PCR experiment. When a protocol is paused during a temperature step, a longer hold time is created for that step.

To pause and then resume a running protocol:

1. Press **PAUSE** on the control panel (Figure 61).

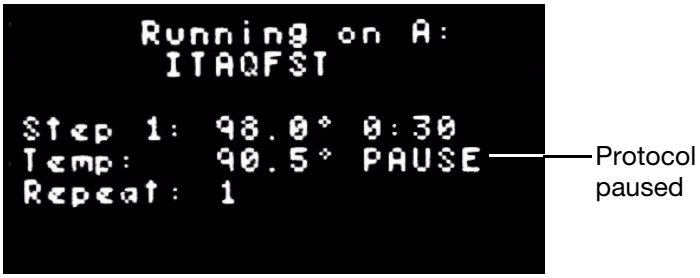

Figure 61. The protocol is paused.

2. To resume the protocol, press **PAUSE** again.

Skipping a Step During the Run

To skip a step while the protocol is running:

1. Press **ENTER** when the step to be skipped is running.

   The thermal cycler skips to the next step in the protocol.

   TIP: The S1000 thermal cycler cannot skip a GOTO repeat, unless the instrument is controlled by a C1000 thermal cycler.

2. Press the right and left arrow key to select **YES** or **No.**
3. Press **ENTER** again to confirm the selection.

Canceling a Run

A protocol may be cancelled while it is running. When a protocol is cancelled, the block immediately stops changing the temperature.

To cancel a running protocol:

1. Press **CANCEL**, then press the arrow key to select **YES** or **No**.

2. Press **ENTER** to confirm the selection, and continue to the next screen. In Figure 62, **YES** is selected.

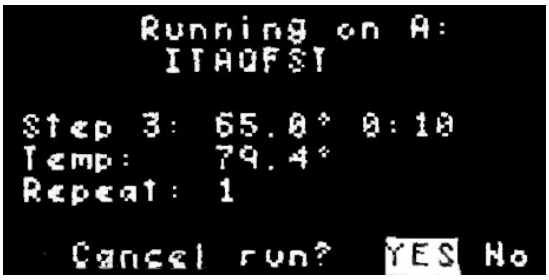

Figure 62. Select YES to cancel a running protocol.

TIP: To cancel a protocol that is running on a dual 48/48 reaction module, press **CANCEL** and then select the block that you want to cancel the run on. Press **ENTER** to confirm the protocol cancellation.

The **PROTOCOL CANCELLED** screen appears. This screen displays the total time that the protocol ran before being cancelled. In Figure 63, the protocol has run 2 minutes and 57 seconds.

Figure 63. The PROTOCOL CANCELLED screen.

3. Press **ENTER** again to return to the main menu

Incubating Samples

Samples may be kept at a constant temperature for any amount of time. The incubation continues indefinitely unless cancelled.

WARNING! Incubating samples for an extended period of time at 4–10°C, particularly in areas of high humidity, can cause excessive moisture condensation around the block.

To start incubating samples:

1. Load your samples into the thermal cycler block and press **INCUBATE**.

   NOTE: For the dual 48 reaction module, the block that contains the samples must first be selected before continuing to the next screen.

2. Select **YES** to use the heated lid during the incubation or **No** to turn off the lid during incubation. Press ENTER to confirm. In Figure 64, **YES** is selected.

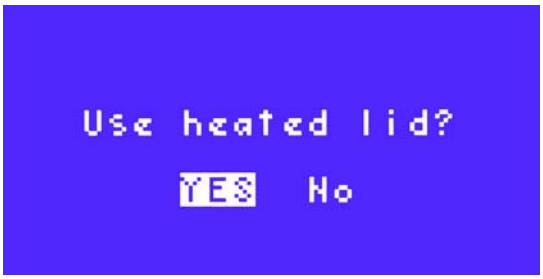

Figure 64. Select YES to use the heated lid during the incubation.

3. If using the heated lid, enter the lid temperature. Press **ENTER** to accept the default lid temperature, or use the numeric keys to type a new lid temperature.

   NOTE: Press **PAUSE** (**.**) key to enter a decimal point. To delete a number, press **CANCEL**.

   In Figure 65, the lid temperature is 100°C.

Figure 65. Enter the lid temperature.

4. Enter the incubation temperature. Press **ENTER** to accept the default incubation temperature of 75°C, or use the numeric keys to type a new incubation temperature between 0 and 100°C.

   NOTE: Press **PAUSE** (**.**) to enter a decimal point. To delete a number, press **CANCEL**.

   In Figure 66, the incubation temperature is 95°C.

Figure 66. Enter the incubation temperature.

5. Press **ENTER** to start the incubation. In Figure 67, the block is incubating at 95.0°C.

Incubation temperature

Figure 67. The incubation temperature is 95.0°C.

TIP: During incubation, use any options in the main menu except **RUN**. You cannot start a run on a block that is running an incubation.

6. To stop an incubation, press **CANCEL**.

7. Select **YES** to stop the incubation or **No** to continue the incubation. Press **ENTER** to confirm the selection and return to the main menu. In Figure 68, **YES** is selected.

Figure 68. Select YES to cancel a protocol.

8. Press **SCREEN** three times to view the incubation parameters summarized in the **LAST RUN** screen (Figure 69).

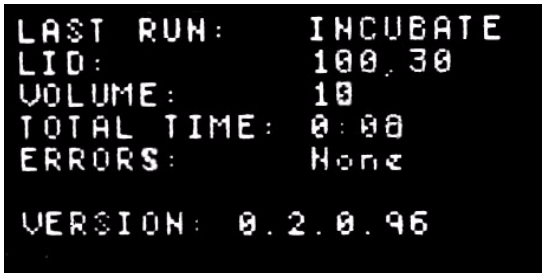

Figure 69. The incubation parameters are summarized in the LAST RUN screen.

4 Managing Protocol Files and Folders

Read this chapter for information on managing protocol files and folders.

Managing Protocol Files and Folders

To manage protocol files and folders, select **FILES** from the main menu to open the file library (Figure 70). The menu of functions in the file library provides options for managing files and folders and changes based on what is selected in the file library.

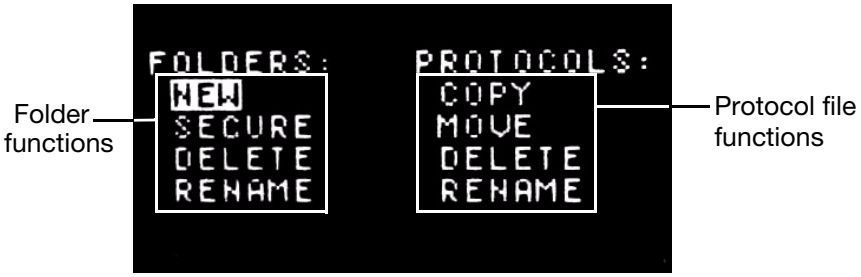

Figure 70. Options for managing protocol files and folders.

Table 11 lists all the functions in the **FILES** option. Protocol and folder names can have a maximum of 8 characters.

Table 11. List of functions in the FILES option

Function	Description
PROTOCOLS	
COPY	Copies an existing protocol file and saves it with a new name
MOVE	Moves a protocol file to another folder
DELETE	Deletes a protocol file
RENAME	Renames a protocol file
FOLDERS	
NEW	Creates a new folder

Table 11. List of functions in the FILES option (continued)

Function	Description
SECURE	Protected files can be deleted and edited once the password protecting them is entered
DELETE	Deletes an empty folder. NOTE: A folder cannot be deleted if it contains protocol files.
RENAME	Renames an existing folder

Copying a Protocol File

To copy a protocol file:

1. Select **FILES** from the main menu.
2. Select **COPY** using the arrow keys (Figure 71), then press **ENTER** to confirm the selection.

   NOTE: The **COPY** function makes a copy of the existing file and requires a new file name for the copied file. You can copy and move secured or preinstalled protocols to your folder.

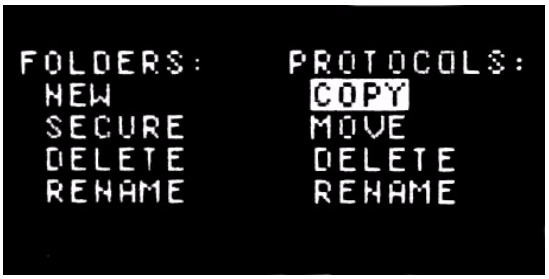

Figure 71. Select COPY using the arrow keys.

3. Using the arrow keys, select the folder that contains the protocol file to be copied, then press the right arrow key to select the appropriate file. Press **ENTER** to continue to the next screen.

   In Figure 72, the **STD2** protocol file is selected in the **MAIN** folder.

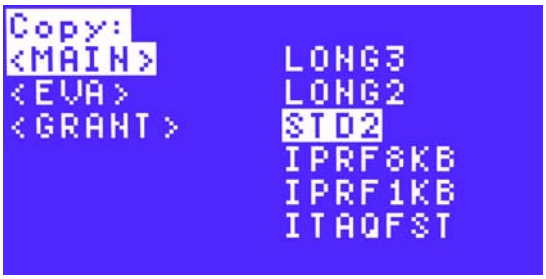

Figure 72. The STD2 protocol file is selected for copying.

4. Using the arrow keys, select the destination folder. In Figure 73, the copied file is placed into the **GRANT** folder. Press **ENTER** to confirm that the protocol was successfully copied.

Figure 73. Select the destination folder.

5. Type a new name for the protocol copied file by pressing the up and down arrows to select letters and the numeric keys to type numbers (Figure 74). Press **ENTER** to accept the selection.

   NOTE: A protocol file name can contain 1–8 characters. The characters are numbers or capital letters. Each protocol name must be unique.

Figure 74. The protocol was successfully copied.

   TIP: After copying the protocol file, make changes to the file by selecting **EDIT** in the main menu (page 22).

Moving a Protocol File

NOTE: If the folder is secure, then you must enter a password to move the file. However, the protocols in a secure folder can be copied to another folder.

To move a protocol file to another folder:

1. Select **Files** from the main menu.
2. Select **MOVE** using the arrow keys (Figure 75), then press **ENTER** to confirm the selection.

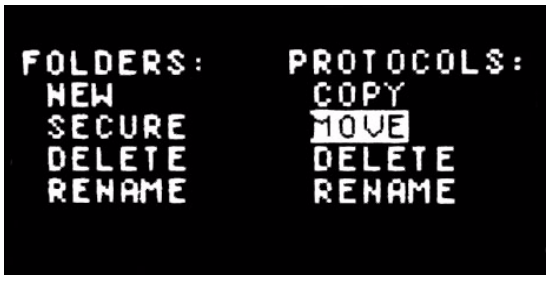

Figure 75. Select MOVE using the arrow keys.

3. Using the arrow keys, select the folder that contains the protocol file to be moved, then press the right arrow key to select the appropriate file. Press **ENTER** to continue to the next screen.
4. Using the arrow keys, select the destination folder. Press **ENTER** to confirm the move.

Deleting a Protocol File

NOTE: If the folder is secure, then you must enter a password to delete the file.

To delete a protocol file:

1. Select **FILES** from the main menu.
2. Select **DELETE** using the arrow keys (Figure 76), then press **ENTER** to confirm the selection.

.

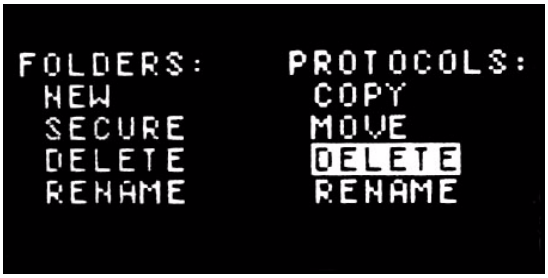

Figure 76. Select DELETE to delete a protocol file.

3. Using the arrow keys, select the folder that contains the protocol file to be deleted, then press the right arrow key to select the appropriate file. Press **ENTER** to continue to the next screen.
4. To delete the file, select **YES** and then press **ENTER** to return to the main menu. Select **No** to cancel the deletion.

Renaming a Protocol File

To rename a protocol file:

1. Select **FILES** from the main menu.
2. Select **RENAME** using the arrow keys (Figure 77), then press **ENTER** to confirm the selection.

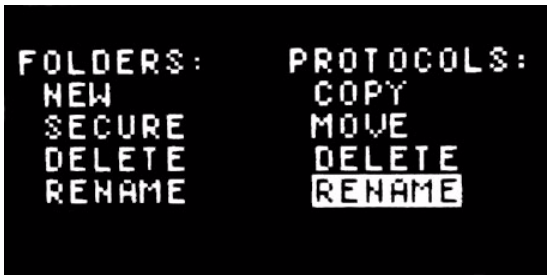

Figure 77. Select RENAME to enter a new protocol name.

3. Using the arrow keys, select the folder that contains the protocol file to be renamed, then press the right arrow key to select the appropriate file. Press **ENTER** to continue to the next screen.
4. Enter a new protocol file name using the up and down arrows to select letters and the numeric keys to type numbers. Then press **ENTER** to accept the new name and return to the **main menu**.

   NOTE: A protocol file name can contain 1–8 characters. The characters are numbers or capital letters. Each protocol name must be unique to all folders on the S1000 thermal cycler.

Creating a New Folder

The S1000 thermal cycler can contain 11 folders in addition to the **MAIN** folder. Protocol files are stored in the **MAIN** folder by default; however, it is highly recommended that files be stored in user-created folders for easy access and ability to password-protect the files.

To create a new folder:

1. Select **FILES** from the main menu.
2. Select **NEW** from the menu using the arrow keys (Figure 78). Press ENTER to confirm the selection.

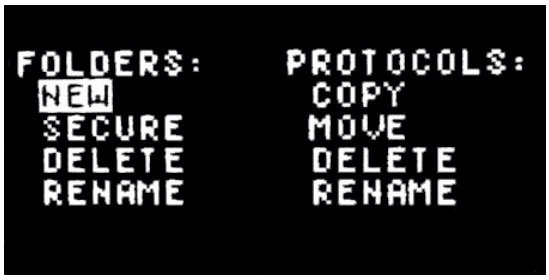

Figure 78. Select NEW to create a new folder.

3. Enter the folder name using the up and down arrows to select letters and the numeric keys to type numbers. Then press **ENTER** to accept the new name and return to the **main menu**.

   NOTE: A folder name can contain 1–8 characters. The characters are numbers or capital letters. Each protocol name must be unique to the thermal cycler.

Securing Files in a Folder

Securing folders with a password prevents other users from editing, deleting, or moving your files from the S1000 thermal cycler.

NOTE: To edit, move, or delete files stored in a secure folder, a password must be entered. However, a password is not required for viewing, copying, or running protocol files that are located in a secure folder.

To secure a folder with a password or to change an existing password:

1. Select **FILES** from the main menu.

2. Select **SECURE** using the arrow keys (Figure 79). Press **ENTER** to confirm the selection and continue to the next screen.

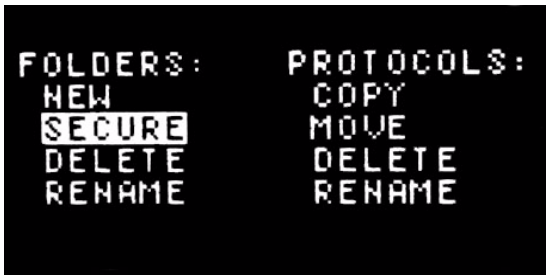

Figure 79. Select SECURE to secure a folder with a password.

3. Select the folder to be secured using the up and down arrows.

   NOTE: To change a password, you need to enter the original password first. If the password is lost, protocols in the secured folder cannot be deleted, moved, or edited. Furthermore, the folder cannot be deleted.

4. Enter a new password using the numeric keys to type numbers. Press **ENTER** to confirm the password, and return to the main menu.

   NOTE: A password can contain one to four numbers, and cannot contain letters.

   TIP: To disable security for a folder, repeat steps 1–4, and specify a blank, new password.

Deleting a Folder

NOTE: A folder that contains protocol files cannot be deleted. Select **VIEW** in the main menu to view the contents of the folder before deleting the folder. Delete or move all protocol files before deleting the folder. Once a folder is deleted, it is permanently removed.

To delete a folder:

1. Select **FILES** from the main menu.
2. Select **DELETE** using the arrow keys (Figure 80), then press **ENTER** to confirm the selection.

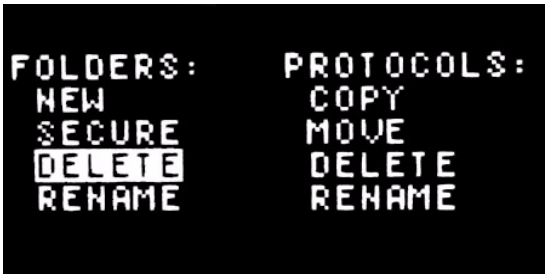

Figure 80. Select DELETE to permanently remove a folder.

3. Using the arrow keys, select the folder to be deleted. Press **ENTER** to continue to the next screen.
4. To delete the folder, select **YES** and then press **ENTER** to return to the main menu. Select **No** to cancel the deletion

Renaming a Folder

NOTE: Renaming a folder does not change the protocol files stored in the folder. To rename a folder that is secure, enter the password before typing the name.

To rename a folder:

1. Select **FILES** from the main menu.
2. Select **RENAME** using the arrow keys (Figure 81), then press **ENTER** to confirm the selection.

   .

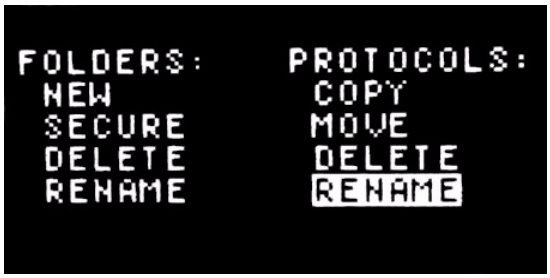

Figure 81. Select RENAME using the arrow keys.

3. Using the arrow keys, select the folder to be renamed. Press **ENTER** to continue to the next screen.
4. Enter the new folder name using the up and down arrows to select letters and the numeric keys to type numbers. Then press **ENTER** to accept the new name and return to the **main menu**.

   NOTE: A folder name can contain 1–8 characters. The characters are numbers or capital letters.

5 Optimizing PCR on the S1000 Thermal Cycler

Read this chapter for information on optimizing PCR on the S1000 thermal cycler.

Optimizing a Protocol for Faster PCR

Optimizing a protocol for faster PCR can reduce the total run time by one-third. In contrast, running the same protocol on a thermal cycler with a faster ramp rate only cuts minutes from the total run time. Optimizing the protocol for speed can also result in better PCR results.

Complete optimization of a protocol involves selecting the appropriate reagents, enzymes, and primers, as well as testing the parameters of the PCR protocol. For more detailed information on optimizing protocols for fast PCR, refer to the following resources:

- **Gene Expression Gateway (www.bio-rad.com/genomics/).** Go to the web site and select *Application* | *Techniques* > *Quantification* > *Fast PCR*
- ***BioRadiations Magazine* volume 118** in PDF. Go to discover.bio-rad.com and search the *Literature* for “Fast PCR”. This magazine includes articles on tips for optimizing the reagents

To optimize a protocol for fast PCR using the S1000 thermal cycler, follow these guidelines:

- **Shorten the denaturing step during cycling**

  The initial denaturation step requires a longer hold time than denaturing steps during each subsequent cycle. This difference is due to the activation of polymerase and to the longer initial DNA template. Once the PCR target is amplified, the amplicons then serve as shorter templates that are easier to denature during cycling.

  To shorten the denaturation step, enter a hold time of 1 sec for PCR products that are less than 500 bp. Then test this shorter hold time to verify that a 1 sec denaturation is sufficient to produce amplicons. Alternatively, add an increment to the denaturation step

to test for the best hold time. See “Adding an Increment to a Temperature Step” on page 19 for detailed instructions.

- **Create a two-step protocol by combining annealing and extension into a single step**

  Most polymerases remain active throughout the typical range of annealing temperatures (55–70°C). Reduce the total run time by creating a two-step protocol that combines the annealing and extension steps into a single step. A two-step protocol can produce a product that is similar to one produced by a three-step protocol for target sequences up to 200 bp.

  To create a two-step protocol, keep the annealing temperature step and omit the extension step. Then adjust the hold time for the annealing step based on the length of the amplicon. Start with a hold time that is 10 sec per 100 bp of the target.

  Alternatively, optimize the annealing temperature using a temperature gradient across the block, and pick the final annealing temperature from the best results of the gradient experiment. See “Optimizing Annealing Temperature Steps with a Gradient” (page 49) as an example. Optimization of the annealing step is critical because it determines the specificity of the reaction. If the annealing temperature is too high, the primers do not anneal easily, and if the annealing temperature is too low, the reaction results in primer mismatches, lower PCR yield, and nonspecific amplification.

- **Optimize temperature steps to minimize the ramping time**

  The larger the temperature difference between two successive steps in a protocol, the longer the time required to reach the next target temperature. Shorten the run time by minimizing the difference between target temperatures in successive steps.

  To minimize target temperature differences, run a temperature gradient by adding a gradient. (See Step 9 on page 14.) Begin by optimizing the difference between the annealing and extension temperatures. Use the results of this gradient experiment to determine the highest possible annealing temperature without sacrificing the PCR yield. See “Optimizing Annealing Temperature Steps with a Gradient” (page 49) as an example. Finally, choose an annealing temperature with the smallest temperature difference from the extension temperature.

- **Minimize the final extension step**

  The final extension step completes the synthesis of amplicons. Optimize this step to obtain a high percentage of complete amplicons at the end of the PCR. During each cycle, the extension step is typically 30 sec. If amplification for 30 sec is sufficient during cycling, then a longer final extension step is unnecessary.

  To minimize the final extension step, choose a hold time between 30 sec and 2 min for targets between 100 and 1,000 bp. Then test the hold time chosen for sufficient amplification. Alternatively, add an increment to the extension step to test for the best hold time.

- **Minimize the number of repeats in a GOTO step**

  Minimize the repeats in the GOTO step to minimize the number of cycles in the protocol. Before adjusting the number of cycles, the approximate concentration of PCR template must be known.

  To approximate the concentration of an unknown template, start with 30 to 45 cycles the first time you run the protocol. Then detect the PCR product in a gel stained with ethidium bromide and estimate the starting concentration. If the concentration is sufficient, shorten the number of cycles by 5 and then run the protocol again. Once the concentration of the target sequence is known, minimize the number of cycles in the protocol until the concentration of the PCR product is too low. Choose the best number of GOTO loops from the results of these reactions.

Optimizing Annealing Temperature Steps with a Gradient

Optimizing the annealing step is critical for an efficient reaction that yields a clean product. Use a gradient to optimize the temperature of the annealing step. In addition, you can use a gradient step to optimize other temperature steps.

Follow these steps as a suggested approach to optimizing the annealing temperature:

1. Calculate the predicted annealing temperature based on the melting temperature (T_m) of the primers and template.

   When the S1000 thermal cycler is connected to a C1000 thermal cycler, use the T_a calculator available on the C1000 cycler to find the T_m of any combination of primers and template.

2. Create a PCR protocol with a gradient step in the annealing step.

   Choose a gradient range that is as wide as possible to test for the optimal annealing temperature, and bracket the calculated T_a by 5–12°C. For example, if the calculated T_a is 55°C, then use a 24°C gradient to bracket that T_a.

   NOTE: The widest possible gradient on the S1000 thermal cycler is 24°C.

3. Choose a high annealing temperature from the results of the gradient PCR run to reduce the chance of nonspecific primer binding. See step 2, above, for details on creating a gradient. In Figure 82, the gradient range is 55–67°C, and the best annealing occurs at 60°C.

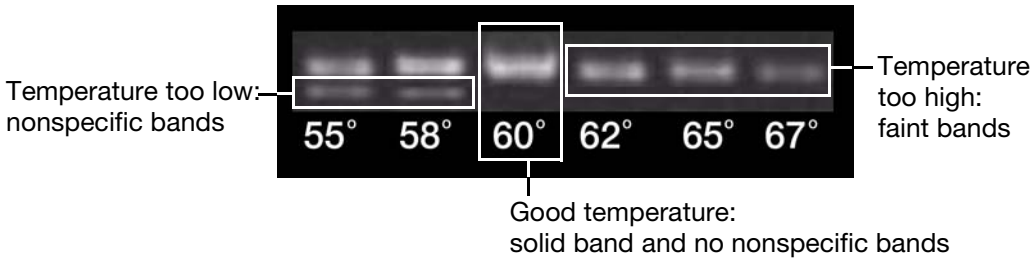

Figure 82. The effect on annealing temperature on the outcome of a PCR run.

   As a starting point, use the highest annealing temperature and subtract 1 or 2°C. Do not use the absolute highest temperature. At higher temperatures, the yield decreases and results in faint bands. Furthermore, at high temperatures, the primers might behave inconsistently from one PCR run to another.

4. (Optional) Run a narrower annealing temperature gradient using the results from the first, wider temperature gradient. Bracket the annealing temperature chosen in step 3 (above) by 5°C. Choose the final annealing temperature from the highest successful temperature in the results of this gradient.

   For example, if the highest successful annealing temperature in step 3 is 60°C, then bracket that temperature with a narrower gradient (±5°C). In this example, the narrow gradient is 55–65°C. Choose the final annealing temperature from a high successful temperature in the gradient experiment as described in step 3 above.

Optimizing PCR With Small Sample Volumes

Running a PCR protocol with a small sample volume requires optimization to prevent evaporation and condensation. Follow these suggestions for optimizing PCR with a reaction volume below 10 µl:

- **Control evaporation with a wax seal**

  A wax, such as Chill-out™ liquid wax, is the best seal for reducing evaporation. Further reduction is possible by using a second seal, such as a cap or film.

- **Reoptimize the annealing temperature to prevent nonspecific priming and increase target amplification**

  When running an established protocol with a smaller sample volume (<10 µl), the annealing temperature may need to be reoptimized. If mispriming or low amplification is observed (and reagent problems have been ruled out), adjust the protocol by optimizing the annealing temperature step using a gradient as described on page 49.

- **Lower the lid temperature to reduce sample loss due to evaporation**

  The heated lid prevents condensation from forming in the microplate or tube, which is critical when the sample volume is small. However, using the same lid temperature with a smaller sample volume can increase evaporation. To prevent evaporation, lower the lid temperature and test the reaction for condensation.

- **Run the reaction in a 384-well reaction module**

  The 384-well block is optimized for small sample volumes and is the best block for this application.

Transferring Protocols from Another Thermal Cycler

To achieve the same results after transferring a PCR protocol to the S1000 thermal cycler from another thermal cycler, the ramp rate may need to be lowered. If identical reactions that are run on a thermal cycler with a slower ramp rate provide the same data, change the ramp rate on the S1000 thermal cycler.

When transferring a protocol to the S1000 thermal cycler, follow these guidelines:

- Match the ramp rate of the other thermal cycler by changing the rate in each relevant step on the S1000 thermal cycler. (See “Changing the Ramp Rate in a Temperature Step” on page 21.) In general, the ramp rate for the annealing step could be lowered when a protocol from a thermal cycler with a slower ramp rate is moved to the S1000 thermal cycler
- Increase the amount of Mg^{++} in the reagents to help the primers anneal when the conditions have changed. Adding Mg^{++} increases the primary product, but may also cause some secondary PCR products
- Adjust the temperature in each step to reoptimize the protocol and the annealing temperature. (See “Optimizing Annealing Temperature Steps with a Gradient” on page 49)

Troubleshooting PCR Problems

This section provides a quick guide for PCR troubleshooting options. For more detailed and extensive troubleshooting, go to the Gene Expression Gateway (www.bio-rad.com/genomics/) and select *Support* > *Amplification Central* > *PCR Doctor*

Use the following suggestions to adjust and reoptimize a reaction that has failed due to the presence of:

- **Nonspecific PCR products, in addition to the target product**

  Nonspecific products result from mispriming. If reagent problems have been ruled out, increase the annealing temperature to increase specificity of primer binding. To find the optimal annealing temperature, use a gradient.

- **Nonspecific PCR products without the target product**

  Nonspecific product with no target production is a result of complete mispriming. If reagent problems have been ruled out, adjust the hold time in the annealing and extension steps to increase specificity of primer binding. Increasing the hold time also provides more time for complete extension.

- **Low yield of the target PCR product**

  Low PCR yield is a result of mispriming, an overly short extension hold time, or too high an annealing temperature. When the low yield is not a result of reagent problems, adjust the protocol.

  Run a touchdown protocol (page 72) to increase amplification of the target product. Alternatively, decrease the annealing temperature or run a gradient to optimize the annealing temperature. (See “Optimizing Annealing Temperature Steps with a Gradient” on page 49).

Selecting Compatible Reaction Vessels and Sealing Options

The composition and thickness of reaction vessels influence the outcome of a reaction. Microplates, tubes, sealers, and caps come in a variety of compositions and colors. Bio-Rad tests the standard supplies for compatibility with the 1000-series thermal cyclers.

Refer to Table 21 on page 78 for a full list of available microplates, tubes, and sealing options that are compatible with 1000-series thermal cyclers. Catalog numbers for these consumables are provided for easy ordering.

Whenever the source or composition of vessels is changed, it is good practice to reoptimize the protocol before running an important experiment.

> NOTE: Sealing wax, such as Chill-out liquid wax, is specifically recommended to seal small sample volumes of less than 10 µl. Wax solidifies at room temperature. Pierce the solid wax with a micropipet tip to remove the sample. For more information about optimizing protocols for small sample volumes, see “Optimizing PCR With Small Sample Volumes” on page 50.

For a full list of available reagents and supplies, refer to the Life Science Research Product catalog or online at discover.bio-rad.com.

6 Advanced Tools and Functions

Read this chapter for information on advanced tools and functions on the S1000 thermal cycler.

- TOOLS options (below)
- Controlling S1000 thermal cyclers with a C1000 thermal cycler (page 61)

TOOLS Options

To see the list of instrument settings and tools:

1. Select **TOOLS** from the main menu. The following functions are available in the **TOOLS** option:
   - **LAST RUN** — to view the last protocol that was run (page 53)
   - **SELF TEST** — to run a self test on the thermal cycler (page 54)
   - **VERSION** — to view the current instrument firmware version (page 55)
   - **NAME** — to enter a name for the thermal cycler (page 56)
   - **DEFAULTS** — to change default lid temperature, “turn off below” feature, and sample volume (page 57)
   - **GRADCALC** — to view a temperature gradient based on user defined parameters (page 58)
   - **CONTRAST** — to change the instrument’s LCD contrast (page 59)
   - **PORT** — to change the port used to control the S1000 thermal cycler remotely (page 60)
2. To return to the main menu, press **ENTER**.

LAST RUN Screen

To see a summary of the last protocol that was run on the S1000 thermal cycler:

1. Select **TOOLS** in the main menu. Press **ENTER** to confirm the selection.

2. Select **LAST RUN** using the arrow keys (Figure 83), and press **ENTER** to confirm the selection.

Figure 83. Select LAST RUN for a summary of the last protocol run on the cycler.

NOTE: For dual 48/48 reaction modules, the **LAST RUN** tool toggles to show the last protocol that was run on each block.

3. The **LAST RUN** screen is displayed (Figure 84). This screen shows a synopsis of the parameters in the last protocol that was run, including:
   - Protocol name
   - Lid temperature during the run
   - **Turn off below** temperature for the lid during the run
   - Sample volume, which determines the temperature control mode during
   - Total run time
   - Number of errors detected during the run
   - Firmware version on the S1000 thermal cycler during the run

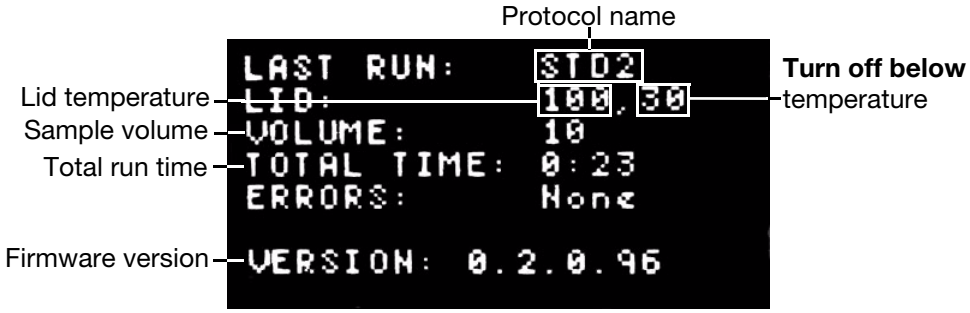

Figure 84. The LAST RUN screen.

4. Press **ENTER** to return to the **TOOLS** option.

Testing the Instrument

The S1000 thermal cycler automatically runs a self test every time it starts a run to check that it is running within specification.

To manually run a self test:

1. Select **TOOLS** in the main menu. Press **ENTER** to confirm the selection.

2. Select **SELFTEST** (Figure 85), and press **ENTER** to continue to the next screen.

Figure 85. Select SELFTEST to manually run a self test.

3. The thermal cycler displays the self testing screen while it runs the test (Figure 86). When the test is successfully completed, the main menu is displayed.

   NOTE: The instrument fans turn on and off during the test.

Figure 86. The self testing screen.

Checking the Firmware Version

To check the firmware version that is currently on the thermal cycler:

1. Select **TOOLS** in the main menu. Press **ENTER** to confirm the selection.
2. Select **VERSION** (Figure 87), and press **ENTER** to continue to the next screen.

Figure 87. Select VERSION from the TOOLS option.

3. The firmware version is displayed on the screen (Figure 88). The header version (**HEADER**) of the reaction module is displayed, which means the firmware expects at least the specified version.

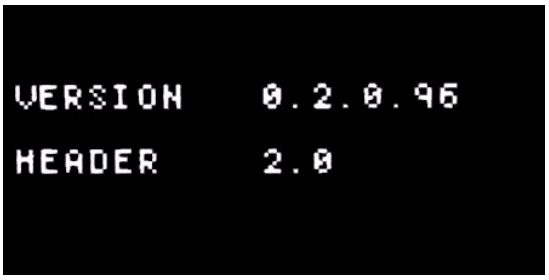

Figure 88. The firmware and header versions are displayed.

4. Press **ENTER** to return to the main menu

Naming the Thermal Cycler

To name or rename the thermal cycler:

1. Select **NAME** using the arrow keys (Figure 89), then press **ENTER**.

   The S1000 thermal cycler name is displayed on the main menu.

Figure 89. Select NAME to enter a new thermal cycler name.

2. Enter the new thermal cycler name by pressing the up and down arrow keys to select a letter and the numeric keys to type a number (Figure 90). Press **ENTER** to accept the selection and continue to the next character in the name.

   NOTE: A cycler name can contain 1–8 characters. The characters can be numbers or capital letters.

Figure 90. Enter the new thermal cycler name one character at a time.

NOTE: The name identifies the S1000 thermal cycler when it is controlled by the C1000 thermal cycler. The thermal cycler name appears in the C1000 thermal cycler instrument tree or in the C1000 Manager software **Detected Instruments** panel on the left side of the screen. If the S1000 thermal cycler is not named, then the S1000 thermal cycler serial number is used.

3. Press **ENTER** to confirm the thermal cycler name (Figure 91) and return to the main menu.

Figure 91. Press ENTER once the thermal cycler name is entered.

Changing the Default Parameters

The following three default parameters can be modified when running a protocol or incubating samples:

- **Lid Target** — sets the default lid temperature for a new protocol. The default temperature is set at 105°C for 96- or dual 48-well reaction blocks and 95°C for 384-well blocks. The new lid temperature can be 0–110°C
- **Turn off below** — when the block is running an infinite hold at a temperature below the **Turn off below** parameter, the lid heater is maintained at 31°C
- **Sample Vol** — sets the default sample volume in a new protocol (see ep 4 on page 13). The sample volume range can be 0–50 µl (or 0–30 µl when using the 384-well reaction modules) and the default parameter is 10 µl. See page 3 for the recommended reaction volume

To view or change the default parameters on the S1000 thermal cycler:

1. Select **DEFAULTS** using the arrow keys (Figure 92). Press **ENTER** to continue to the next screen.

Figure 92. Select DEFAULTS to change the default parameters on the thermal cycler.

2. Press the up and down arrow keys to select the default setting to be modified.
   Figure 93 shows the default parameters for the S1000 thermal cycler.

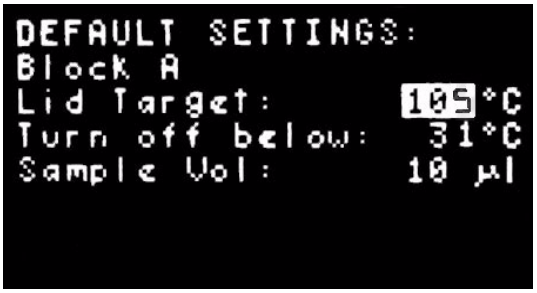

Figure 93. The default parameters for the S1000 thermal cycler.

3. Use the numeric keys to enter the new parameter. Press **ENTER** to confirm the number and move to the next parameter to be modified.
4. To save the parameters, select **YES** and press **ENTER**. To reject changes and return to the main menu, use the right arrow key to select **No** and press **ENTER**.

Viewing a Temperature Gradient

In the S1000 thermal cycler, the temperature gradient is distributed from the front (row H; coolest temperatures) to the back (row A; hottest temperatures) of the block.

To review a temperature gradient to determine the temperature in each row of the block:

1. Select **TOOLS** from the main menu.
2. Select **GRADCALC** using the arrow keys (Figure 94), and press **ENTER** to continue to the next screen.

Figure 94. Select GRADCALC to view the temperature in each row of the block.

3. Enter the lower temperature in the gradient using the numeric keys. The limit is 30.0–99.0°C. Press **CANCEL** (**.**) to enter a decimal point.

   In Figure 95, the lower temperature is 65°C.

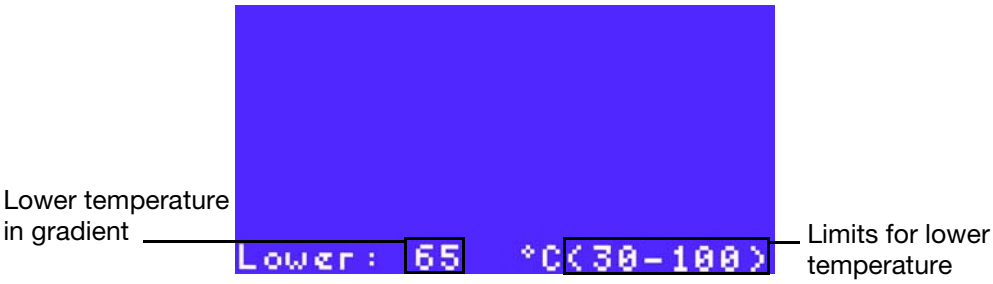

Figure 95. Enter the lower temperature in the gradient.

4. Enter the upper temperature in the gradient using the numeric keys. Press **CANCEL** (**.**) to enter a decimal point. The upper temperature must be greater than the lower temperature, and must be within 24.0°C of the lower temperature.

In Figure 96, the upper temperature is 75°C.

Figure 96. Enter the upper temperature in the gradient.

5. The temperature gradient is displayed in each row of wells in the block.

   In Figure 97, the temperature in row D of this 96-well block is 71.4°C.

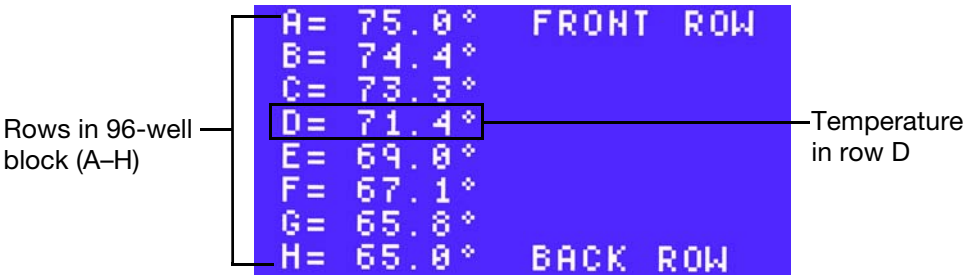

Figure 97. The temperature gradient is displayed in each row of wells in the block.

NOTE: The temperature in the middle rows is estimated. The estimate is based on the temperatures in the front and back rows and on the number of rows in the block.

Changing the Screen View

To change the contrast of the LCD and improve the visibility of characters displayed on the screens, use the following instructions:

1. Select **TOOLS** from the main menu.
2. Select **CONTRAST** using the arrow keys (Figure 98), and press **ENTER** to continue to the next screen.

Figure 98. Select CONTRAST to change the contrast of the LCD.

3. Adjust the contrast using the right and left arrow keys.

   NOTE: Press the left arrow key to decrease (–) the contrast and the right arrow key to increase (+) the contrast.

In Figure 99, the contrast is increased.

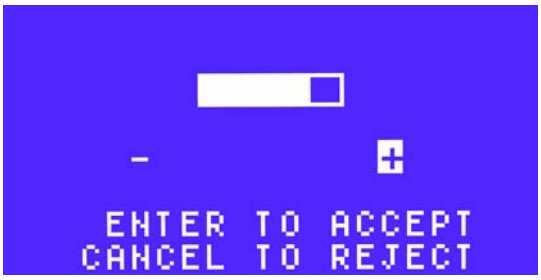

Figure 99. Increase the contrast using the right arrow key.

4. To accept the changes and return to the **TOOLS** options, press **ENTER**. To reject the changes and return to the original contrast, press **CANCEL**.

Setting Up the S1000 Cycler For Remote Control

To set up the S1000 thermal cycler for remote control:

1. Select **TOOLS** to instruct the thermal cycler to list the data transfer ports.
2. Select **PORT** (Figure 100) and press **ENTER** to continue to the next screen.

Figure 100. Select PORT to set up the S1000 thermal cycler for remote control.

3. Select a port from the list of all ports on the thermal cycler (Figure 101), and press **ENTER** to return to the **TOOLS** option.

Figure 101. Select a port from the list of all ports on the thermal cycler.

Controlling S1000 Thermal Cyclers with a C1000 Thermal Cycler

The S1000 thermal cycler can be run in stand-alone single instrument, stand-alone multi-instrument, or software-controlled multi-instrument configuration. In stand-alone multi-instrument configuration, up to **three** S1000 thermal cyclers can be run under the control of a C1000 thermal cycler. Each S1000 thermal cycler can be connected to the C1000 thermal cycler through the USB A port of the C1000 thermal cycler.

When connected to a C1000 thermal cycler, the S1000 thermal cyclers can be controlled by either the control panel on the C1000 thermal cycler or the C1000 Manager software.

Connecting S1000 Cyclers Directly to a C1000 Cycler

To connect up to three S1000 thermal cyclers directly to a C1000 thermal cycler, follow these instructions:

1. Plug a high quality, shielded USB cable into the USB B port on the back of the S1000 thermal cycler.

   For the USB cable part number, see “Accessories for the 1000-Series Thermal Cyclers” on page 78.

2. Plug the other side of the USB cable into a USB A port on the back of the C1000 thermal cycler.

   The C1000 thermal cycler instrument detects the attached S1000 thermal cycler.

3. Repeat steps 1 and 2 to connect up to three S1000 cyclers directly to the same C1000 thermal cycler.

4. Open the MAIN screen or instrument tree on the C1000 thermal cycler, or in the C1000 Manager software if the C1000 thermal cycler is connected to a computer.

5. Select the S1000 thermal cycler by serial number or name.

   NOTE: If the S1000 thermal cycler instrument has a name, then the name is displayed instead of the serial number.

Operating the S1000 Thermal Cycler While Under the Control of the C1000 Cycler

When the S1000 thermal cycler is under the control of the C1000 thermal cycler, it is in “semi-lock down mode”. In this mode, the S1000 thermal cycler does not respond when control panel keys are pressed. However, the following keys function on the control panel:

- **SCREEN** — to access the running, graphical, and time remaining screens
- **PAUSE** — to temporarily stop a protocol that is currently running on the S1000 thermal cycler. This function is active when an individual protocol screen is being displayed
- **CANCEL** — to cancel a protocol that is currently running on the S1000 thermal cycler. This function is active when an individual protocol screen is being displayed
- **ENTER** — to begin a run that has been remotely sent from the C1000 Manager software
- **ENTER** — to skip a step. This function is active when an individual protocol screen is being displayed

Controlling Thermal Cyclers Using the C1000 Manager Software

In a software-controlled multi-instrument configuration, the C1000 Manager software controls up to 32, 1000-series cyclers at once from a single computer. Protocols can be run on individual or multiple blocks, either independently or simultaneously.

> NOTE: In a software-controlled configuration, the S1000 thermal cycler is not directly controlled by the C1000 Manager software. The S1000 thermal cycler must first be connected to a C1000 thermal cycler that is attached to a PC computer running the C1000 Manager software. See “Connecting S1000 Cyclers Directly to a C1000 Cycler” on page 61.

For detailed instructions on using the C1000 Manager software to control S1000 thermal cyclers, see the C1000 thermal cycler instruction manual.

7 Maintenance and Troubleshooting

Read this chapter for information on maintaining the S1000 thermal cycler and troubleshooting problems on the thermal cycler.

Cleaning and Maintaining the S1000 Thermal Cycler

The S1000 thermal cycler requires little maintenance for proper operation and precise thermal control. However, with long and constant use, the thermal cycler requires some cleaning and other maintenance. Information on cleaning the thermal cycler base and reaction module is included in this chapter. In addition, instructions on replacing the fuses are provided.

TIP: For robotic installations where many instruments run constantly, perform a regular check for dust, spills, and debris that could interfere with optimal instrument performance.

Cleaning the S1000 Thermal Cycler

The S1000 thermal cycler should be cleaned on a regular schedule to remove any debris or dirt that might interfere with proper function. Clean the base to prevent damage to the air intake or reaction module bay.

NOTE: For instructions on handling and cleaning radioactive or biohazardous materials, consult the guidelines for radiation safety and biosafety provided by your institution. These guidelines include cleaning, monitoring, and disposal methods for hazardous materials.

To clean the thermal cycler base, follow the instructions below, paying careful attention to the warnings:

WARNING! To prevent electrical shock, always turn off and unplug the instrument before cleaning it.

- **Clean the air vents.** Remove dust with a soft brush, damp cloth, or vacuum cleaner. Remove any heavy dust that is deep in the vents with a vacuum cleaner. Cleaning the vents allows sufficient air flow for precise thermal control during a run

- **Clean the control panel.** Remove debris on the control panel with a soft cloth and mild soap solution. Cleaning the panel prevents debris that may obscure the display

  WARNING! Do not use abrasive detergents or rough material since they scratch the control panel.
- **Clean the reaction module bay.** Clean with a damp soft cloth to remove debris and spilled liquids. Cleaning the bay allows precise heating and cooling of the reaction block

  WARNING! Never use cleaning solutions that are corrosive to aluminium. Avoid scratching the surface of the bay, which interferes with precise thermal control.

  WARNING! Never pour water or other solutions in the reaction module bay. Wet components can cause electrical shock when the thermal cycler is plugged in.
- **Clean the outside case of the thermal cycler base.** Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and remove the residue completely. Cleaning the outside case prevents corrosion

Cleaning the Reaction Modules

Clean the reaction modules of the S1000 thermal cycler on a regular schedule to prevent reagents from accumulating and interfering with the ability of the reaction block to change temperature quickly.

To clean the reaction module, follow these instructions, paying careful attention to the warnings:

WARNING! To prevent electrical shock, always remove the reaction module from the thermal cycler base before cleaning it.

- **Clean the cooling fins.** Remove dust from the cooling fins with a soft brush or damp cloth. Remove any heavy dust that is deep in the fins with a vacuum cleaner. Use water and a soft cloth to remove debris that is stuck to the fins. Avoid scratching the surface. Never use cleaning solutions that are corrosive to aluminum, such as bleach or abrasive cleansers. If needed, use a mild soap solution and rinse well to remove the residue completely. Cleaning the fins improves precise sample heating and cooling
- **Clean the outside cover of the reaction block**. Use a soft cloth and water to remove debris from the outer block

  WARNING! Never clean the block with strong alkaline solutions (strong soap, ammonia, or high-concentration bleach). Never use corrosive or abrasive cleaning solutions. These cleaning agents can damage the block and prevent precise thermal control.
- **Clean the block wells.** Clean spills immediately to prevent them from drying inside wells. Use disposable plastic pipets with water (recommended), 95% ethanol, or a 1:100 dilution of bleach in water. Always rinse the wells with water several times to remove all traces of ethanol, bleach, or soap

  WARNING! If left in the block wells, bleach, ethanol, or soap could corrode the block and/or destroy tubes and microplates during a run. Always rinse the block well after cleaning it with any solution other than water.
- **If oil is used, the wells must be cleaned thoroughly and often**. Use of oil in the wells is not recommended. Clean the oil when it is discolored or contains dirt. Use a solution of 95% ethanol to clean oil. Do not allow oil to build up in the block

  WARNING! Never heat the block after adding a cleaning solution. Heating the block with cleaning solution damages the block, lid, and thermal cycler base.
- **Clean the inner of the reaction module.** Use a soft cloth and water to remove debris and solutions from the inner lid surface. Never use abrasive detergents or rough material

that scratch the surface. Cleaning the inner lid improves precise sample heating and cooling

- **Clean the outer lid surface of the reaction module.** Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and rinse the surface with a damp cloth. Cleaning the cover prevents corrosion

Maintaining Sufficient Air Flow

The S1000 thermal cycler requires sufficient air flow to precisely heat and cool to the correct target temperature. If the flow of air is blocked the thermal cycler cannot ramp to the correct temperature in the specified time. This section includes instructions for testing the air flow and provides suggestions for fixing low or warm air flow.

Testing for Sufficient Air Flow

The air flow is sufficient when the thermal cycler heats and cools to the correct target temperatures promptly. When the S1000 thermal cycler is first set up in a new location, use the following steps to determine the presence of sufficient air flow:

1. Set up the instrument in the location where it is going to be used, then turn the power on.
2. Adjust the local environment for typical conditions.

   Turn on any nearby equipment, such as fans. Also open any window blinds to reproduce typical conditions during a run. If more than one thermal cycler is in the area, run a protocol on all the thermal cyclers at the same time.
3. Run a typical PCR protocol for 30 min.

   To run a protocol, samples are not required; however, an empty microplate or tubes should be included. The lid does not heat correctly if it touches the hot block of the reaction module.
4. Measure the air temperature at the air intake vents of all the thermal cyclers.

   If the air intake temperature increases above 31°C, see “Fixing Insufficient Air Flow” to ensure sufficient air flow.

Fixing Insufficient Air Flow

If the air temperature near the thermal cycler is above 31°C, make one or more of the following changes to increase the flow of cooler air around the thermal cycler:

- Adjust air conditioning to lower the ambient air temperature
- Move the thermal cycler to another location
- Provide more space around the S1000 thermal cycler and between adjacent instruments. Arrange instruments so that the warm exhaust air from one instrument does not enter the air intake vents of another
- Shield the thermal cycler from heat sources, such as radiators, other heat-producing instruments, and bright sunlight

Replacing Fuses

Fuses on the S1000 thermal cycler are designed to blow in case of severe power surges or other causes of electrical short. This process protects both the user and the instrument from excessive electric charge. Fuses on the S1000 thermal cycler rarely need to be replaced. However, some institutions prefer to replace fuses on a regular basis to maintain uninterrupted operation.

If the thermal cycler does not turn on, first check that the power cord is plugged in to a functioning power source. Also, check that the power cord and power source are within the specifications for this instrument. To replace the power cord, contact Bio-Rad Technical Support. For contact information, see “Bio-Rad Laboratories Resources” on page iii.

Finally, check that the fuses are intact. The S1000 thermal cycler runs with two fuses (Figure 102). To remove and check the fuses, follow these steps:

WARNING! To prevent electrical shock, always turn off and unplug the instrument from an electrical outlet before checking the fuses.

1. Use a small coin to unscrew the fuse drawer.

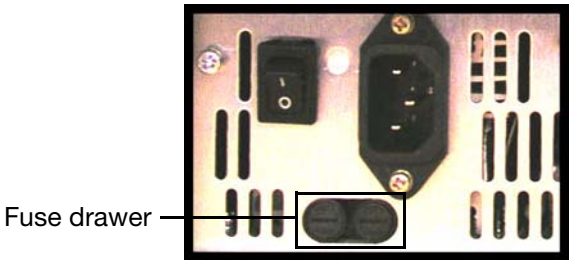

Figure 102. The fuse drawer on the back of the S1000 thermal cycler.

2. Pull out the fuse drawer and remove each fuse.
3. If a fuse is damaged, replace it with the correct fuse, and close the drawer.

   A bad fuse shows a break or burned spot in the metal. A good fuse has intact metal.

Troubleshooting Error Messages on the S1000 Cycler

In general, when the S1000 thermal cycler displays a warning or error message, the instructions for fixing the problem are contained in the message.

Warning and Error Messages

A warning or error message is displayed when external power fails during a run (Figure 103). This error message displays what protocol was running, when the protocol stopped due to the power failure, and the block temperature when the thermal cycler resumed the run.

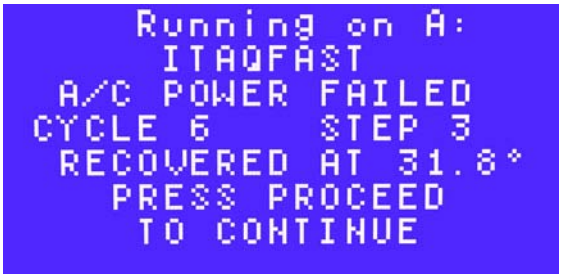

Figure 103. An error message alerting that the protocol was interrupted.

NOTE: A power failure can change the outcome of a PCR run. The hold time for the step that was running when the power failed is lengthened, causing the sample to deviate from the target temperature until the power resumes

The S1000 thermal cycler tracks errors that occur during a run. After a run, all messages are displayed. For example, when a run is cancelled, the **PROTOCOL CANCELLED** screen is displayed as shown in Figure 104.

Figure 104. The PROTOCOL CANCELLED screen.

To clear a message or error and continue to the next screen, press **ENTER**. When all messages are cleared, the S1000 thermal cycler returns to the **main menu**.

The total number of error messages that occur during a run is displayed on the **LAST RUN** screen (page 53). However, there is no process in the S1000 thermal cycler to open these error messages after they are closed at the end of a run. If you want to track and log error messages, run the S1000 thermal cycler under the control of a C1000 or a robotic system that can retrieve a list of errors in the system and run logs.

Error messages are recorded in the **Run Logs** as listed in Table 12.

TIP: The S1000 thermal cycler also tracks errors and system messages for all attached S1000 thermal cycler thermal cycler.

several error messages indicate problems that can be resolved or that might not change the results of your PCR. The table below contains a list of error messages and possible solutions:]

Table 12. Warning and error message solutions

Message	Cause
A/C POWER FAILED POWER OUTAGE DURING CYCLE X STEP Y RESTARTED AT ZZ.Z TO CONTINUE PRESS ENTER	Displayed when a machine running a protocol has been turned off, either intentionally or due to a power outage, and then turned on again.
PLEASE RESTART CYCLER PLEASE CALL BIO-RAD FOR SERVICE BLOCK OVERHEATED	Reaction module has exceeded maximum temperature of 107.5°C or sensor has a malfunction and is not measuring temperature accurately. Protocol terminated.
PLEASE RESTART CYCLER HEATSINK OVERHEATED PLEASE CALL BIO-RAD FOR SERVICE	Heatsink temperature has exceeded 75°C. Protocol terminated.
PLEASE RESTART CYCLER SYSTEM OVERHEATED PLEASE CALL BIO-RAD FOR SERVICE	Amplifier 1 temperature has exceeded 85°C. Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE ALL BLOCK SENSORS FAILED	All block sensors have failed (see below for failure criteria). Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE POWER SUPPLY OVERHEATED	Power Supply temperature has exceeded 85°C. Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE HEATED LID FAILED PROTOCOL CANCELLED	Lid sensor has failed during lid preheat. Protocol terminated.
PLEASE CHECK AIRFLOW HTSINK OVERHEATING PLEASE CALL BIO-RAD FOR SERVICE	Heatsink has exceeded 70°C. System beeps and displays error.
PLEASE CHECK AIRFLOW SYSTEM OVERHEATING PLEASE CALL BIO-RAD FOR SERVICE	Amp temp has exceeded 80°C. System beeps and displays error.
PLEASE CHECK AIRFLOW PS OVERHEATING PLEASE CALL BIO-RAD FOR SERVICE	Power Supply temperature has exceeded 80°C. System beeps and displays error.
PLEASE CALL BIO-RAD FOR SERVICE SLOW BLOCK CYCLING	Block failed to achieve target in the estimated time.
PLEASE CALL BIO-RAD FOR SERVICE SLOW LID CYCLING	Lid failed to achieve target in the estimated time.

Table 12. Warning and error message solutions

Message	Cause
PLEASE CALL BIO-RAD FOR SERVICE SLOW GRADIENT	Block failed to achieve gradient in the estimated time.
PLEASE CALL BIO-RAD FOR SERVICE HEATED LID FAILED	(singles only) If the right and left lid heater channels deviate from each other by more than 5°C the lid is shut off.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 0 FAILED	Block sensor 0 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 1 FAILED	Block sensor 1 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 2 FAILED	Block sensor 2 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 3 FAILED	Block sensor 3 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 4 FAILED	Block sensor 4 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK SENSOR 5 FAILED	Block sensor 5 has failed* and the protocol was terminated.
PLEASE CALL BIO-RAD FOR SERVICE LEFT LID SENSOR FAILED	Left lid sensor has failed*. If a dual, protocol terminated. If a single and BOTH lid sensors failed, protocol terminated and block sent to 4°C.
PLEASE CALL BIO-RAD FOR SERVICE RIGHT LID SENSOR FAILED	Right lid sensor has failed*. If a dual, protocol terminated. If a single and BOTH lid sensors failed, protocol terminated and block sent to 4°C.
PLEASE CALL BIO-RAD FOR SERVICE LEFT HEATSINK SENSOR FAILED	Left heatsink sensor has failed*, system using average of amplifier temperatures to continue.
PLEASE CALL BIO-RAD FOR SERVICE RIGHT HEATSINK SENSOR FAILED	Right heatsink sensor has failed* system using average of amplifier temperatures to continue.
PLEASE CALL BIO-RAD FOR SERVICE LID OVERHEATED AND WAS SHUT OFF	(duals only) Lid has overheated and has been shut off and protocol has been terminated.
PLEASE CALL BIO-RAD FOR SERVICE AMP1 TEMP SENSOR FAILED	Amplifier temperature sensor 1 has failed*.

Table 12. Warning and error message solutions

Message	Cause
PLEASE CALL BIO-RAD FOR SERVICE POWER SUPPLY SENSOR FAILED	Power Supply sensor has failed*.
PLEASE CALL BIO-RAD FOR SERVICE BLOCK POWER FAILURE PROTOCOL CANCELLED	Power to block is out of range.
PLEASE CALL BIO-RAD FOR SERVICE LOGIC POWER FAILURE	Logic power sensor is out of bounds.
PLEASE CALL BIO-RAD FOR SERVICE BASE POWER FAILURE PROTOCOL CANCELLED	Base power sensor is out of bounds. Protocol cancelled.
PLEASE CALL BIO-RAD FOR SERVICE AMP2 TEMP SENSOR FAILED	Amplifier temperature sensor 2 has failed*.
BLOCK MISSING PROTOCOL CANCELLED PLEASE CALL BIO-RAD FOR SERVICE	Reaction module has been removed. Protocol terminated.
PLEASE CALL BIO-RAD FOR SERVICE MEMORY CORRUPT PROTOCOLS MAY BE LOST	Protocol storage memory has been corrupted.
PLEASE RESTART CYCLER BAD REACTION MODULE PLEASE CALL BIO-RAD FOR SERVICE	Unable to read information from reaction module properly.
PLEASE RESTART CYCLER INCORRECT CHECKSUM PLEASE CALL BIO-RAD FOR SERVICE	Information read from reaction module appears incorrect.
PLEASE RESTART CYCLER BLOCK POWER SHUT OFF PLEASE CALL BIO-RAD FOR SERVICE	There was a problem with the block and power was shut off
PROTOCOL CANCELLED SAMPLES COOLED AT 4C BLOCK SENSOR FAILED AT CYCLE X, STEP Y PLEASE RESTART CYCLER PLEASE CALL BIO-RAD	One of the block sensors in a single block has failed. The system has cancelled the protocol at step x, cycle y and sent the block to 4°C to preserve the samples.

The error message may instruct the user to contact Bio-Rad. In this event, call the nearest Bio-Rad Laboratories Technical Support team (see “Bio-Rad Laboratories Resources” on page iii for more detail).

*Sensor Failure means that the sensor was deemed short, open or had changed more than 3°C in a 50 msec period and that this condition was present for more than 2 sec.

If two or more block sensors fail (or both lid sensors fail), the protocol is terminated and the block is sent to 4°C to preserve samples.

Appendix A: Preinstalled Protocols

Standard Protocols

The S1000 thermal cycler is packaged with two standard protocols that run two-step and three-step PCR with a standard DNA polymerase. Use these protocols to begin running PCR with new primers and DNA template, or copy them to begin writing a new protocol. Table 13 lists the parameters in each standard protocol.

Table 13. Standard protocols

	STD2		**STD3**	
	Standard Two-Step Protocol		Standard Three-Step Protocol	
Step	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**
1	95	3:00	95	3:00
2	95	0:30	95	0:30
3	65	0:30	55	0:30
4	GOTO 2	29x	72	0:30
5	72	7:00	GOTO 2	29x
6	12	Infinite hold ∝	72	1:00
7	END		12	Infinite hold ∝
8			END	

Touchdown Protocol

A touchdown protocol tests for the best annealing temperature for a specific primer-template pair. Choose this protocol to test a new set of primers and DNA template for the optimal annealing temperature. Table 14 lists the parameters of the preinstalled touchdown protocol.

Table 14. Touchdown protocol

	TCHDOWN	
	To Identify the Optimal Annealing Temperature	
Step	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**
1	95	3:00
2	95	0:30
3	60 (increment at –0.5°C/ cycle)	0:30
4	72	0:30
5	GOTO 2	29x
6	95	0:30
7	45	0:30
8	72	0:30
9	GOTO 6	29x
10	72	7:00
11	12	Infinite hold ∝
12	END	

Optimized Protocol Using iTaq™ Polymerase

iTaq DNA polymerase is an antibody-mediated hot-start polymerase that is suitable for both PCR and real-time. iTaq polymerase is designed to be activated during the first step at 98°C and to amplify small to medium-size templates. The iTaq protocol runs PCR using the optimal parameters for this polymerase and associated buffers. Table 15 lists the parameters of the iTaq protocol.

Table 15. iTaq protocol

	iTAQ-FST	
	For Fast PCR	
Step	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**
1	98	0:30
2	92	0:01

Table 15. iTaq protocol (continued)

3	70	0:10
4	GOTO 2	29x
5	72	0:30
6	END	

Optimized Protocols Using iProof™ Polymerase

iProof DNA polymerase is a high-fidelity polymerase that is designed to quickly and precisely amplify long targets using a proofreading enzyme combined with a DNA binding protein. Three protocols optimized using the iProof DNA polymerase are preinstalled on the S1000 thermal cycler. These protocols include optimal parameters for the iProof enzyme and associated buffers, including an initial 95°C step to activate the enzyme and a final long extension step. Each of these protocols is adjusted for a distinct range of target sizes. Table 16 lists the parameters in each protocol

Table 16. iProof protocol

	IPRF1KB		**IPRF8KB**		**IPRF15KB**	
	For Fast Amplification of Targets Less Than or Equal to 1 kb Targets		To Amplify Targets Less Than or Equal to 8 kb		To Amplify Targets Less Than or Equal to 15 kb	
Step	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**
1	98	0:30	98	0:30	98	0:30
2	98	0:05	98	0:05	98	0:05
3	60	0:10	60	0:10	60	0:10
4	72	0:30	72	4:00	72	7:30
5	GOTO 2	29x	GOTO 2	29x	GOTO 2	29x
6	72	5:00	72	5:00	72	5:00
7	12	Infinite hold ∝	12	Infinite hold ∝	12	Infinite hold ∝
8	END		END		END	

Optimized Protocol Using the iScript™ Reverse Transcriptase

Reverse transcription protocols are used to amplify DNA from an RNA template. Table 17 lists the parameters of the protocol optimized using the iScript reverse transcriptase.

Table 17. iScript protocol

	ISCRIPT	
	To Amplify DNA from an RNA Template	
Step	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**
1	25	0:30
2	42	30:00
3	85	5:00
4	12	Infinite hold ∝
5	END	

Nested Primer Protocols

Nested primers amplify a specific DNA sequence from a large, complex DNA template such as genomic DNA. Table 18 lists the parameters in each protocol.

Table 18. Nested primer protocol

	NESTPR2		**NESTPR3**	
	Two-Step Protocol Using Nested Primers		Three-Step Protocol Using Nested Primers	
Step	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**	**Target Temperature (°C) or GOTO Step**	**Time (min:sec) or Repeats**
1	95	4:00	95	0:30
2	95	0:30	95	0:30
3	65	0:30	55	0:30
4	GOTO 2	39x	72	0:30
5	72	7:00	GOTO 2	39x
6	12	Infinite hold ∝	72	7:00
7	95	0:30	12	Infinite hold ∝
8	65	0:30	95	0:30
9	GOTO 7	39x	55	0:30
10	72	7:00	72	0:30
11	12	Infinite hold ∝	GOTO 8	39x

Table 18. Nested primer protocol (continued)

12	END		72	7:00
13			12	Infinite hold ∝
14			END	

Appendix B: Ordering Information

Components of Bio-Rad's 1000-Series Thermal Cyclers

Table 19 lists the components of the 1000-series instruments and software. Catalog numbers are included for easy ordering.

Table 19. Catalog numbers for the 1000-series instruments and software

Catalog Number	Description
Thermal Cycler Bases and Reaction Modules	
184-2000	S1000 thermal cycler base
184-1000	C1000 thermal cycler base
184-0096	96-well fast reaction module
184-0048	Dual 48/48 fast reaction module
184-0384	384-well reaction module
185-2096R	S1000 thermal cycler with 96-well fast reaction module and sample reagents
185-2048R	S1000 thermal cycler with dual 48/48 fast reaction module and sample reagents
185-2384R	S1000 thermal cycler with 384-well reaction module and sample reagents
185-1096R	C1000 thermal cycler with 96-well fast reaction module and sample reagents
185-1048R	C1000 thermal cycler with dual 48/48 fast reaction module and sample reagents
185-1384R	C1000 thermal cycler with 384-well reaction module and sample reagents
Real-Time Detection Modules (Compatible with the C1000 thermal cycler base)	
184-5096	CFX96™ optical reaction module
184-5385	CFX384™ optical reaction module
Software	
184-4000	C1000 Manager software

Table 19. Catalog numbers for the 1000-series instruments and software (continued)

Catalog Number	Description
184-5000	CFX Manager software
184-5001	CFX Manager software, security edition, 1 user license
184-5005	CFX Manager software, security edition, 5 user licenses
184-5010	CFX Manager software, security edition, 10 user licenses

Accessories for the 1000-Series Thermal Cyclers

Table 20 lists accessory parts for the 1000-series instruments, including product descriptions and catalog numbers.

Table 20. Catalog numbers of accessories for the 1000-series thermal cyclers

Catalog Number	Description
184-8000	Shielded USB cable
184-9000	Tube frame for providing structural support for one or a few tubes
184-1001	1000-series connectivity kit, includes optical mouse, mouse pad, and USB key

Table 21 lists standard consumables that have been tested for compatibility with the 1000-series thermal cyclers.

Table 21. Catalog numbers of microplates, tubes, and sealing options that are compatible with 1000-series thermal cyclers

Catalog Number	Description
Tubes	
TFI-0201	0.2 ml tubes with flat caps, natural, 1,000
TWI-0201	0.2 ml tubes with domed caps, natural, 1,000
TLS-0801*	Low-profile 0.2 ml 8-tube strips without caps, natural, 120 strips (960 tubes)
TBS-0201*	Full-height 0.2 ml 8-tube strips without caps, natural, 125 strips (1,000 tubes)
48-Well Plates	
MLL-4801*	Multiplate™ low-profile 48-well unskirted PCR plates, natural, 50
MLP-4801*	Multiplate full-height 48-well unskirted PCR plates, natural, 50
96-Well Plates	
HSP-9601*	Hard-Shell® low-profile 96-well skirted PCR plates, white shell, clear well, 50
HSS-9601*	Hard-Shell full-height 96-well semi-skirted PCR plates, clear shell, white well, 50
MLL-9601*	Multiplate low-profile 96-well unskirted PCR plates, natural, 25
MLP-9601*	Multiplate full-height 96-well unskirted PCR plates, natural, 25

Table 21. Catalog numbers of microplates, tubes, and sealing options that are compatible with 1000-series thermal cyclers (continued)

Catalog Number	Description
384-Well Plates	
HSP-3801*	Hard-Shell 384-well skirted PCR plates, clear shell, clear well, 50
Sealers	
TCS-0803	Optical flat 8-cap strips, for 0.2 ml tubes and plates, ultraclear, 120 strips
TCS-0801	Domed 8-cap strips, for 0.2 ml tubes and plates, 130 strips
MSB-1001	Microseal® 'B' adhesive seals, optically clear, 100
MSA-5001	Microseal 'A' sealing films, 100
MSF-1001	Microseal 'F' sealing foil, 100

* Other color options or packaging sizes available. Check Bio-Rad catalogs for a complete list of color, packaging, and barcode options.

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