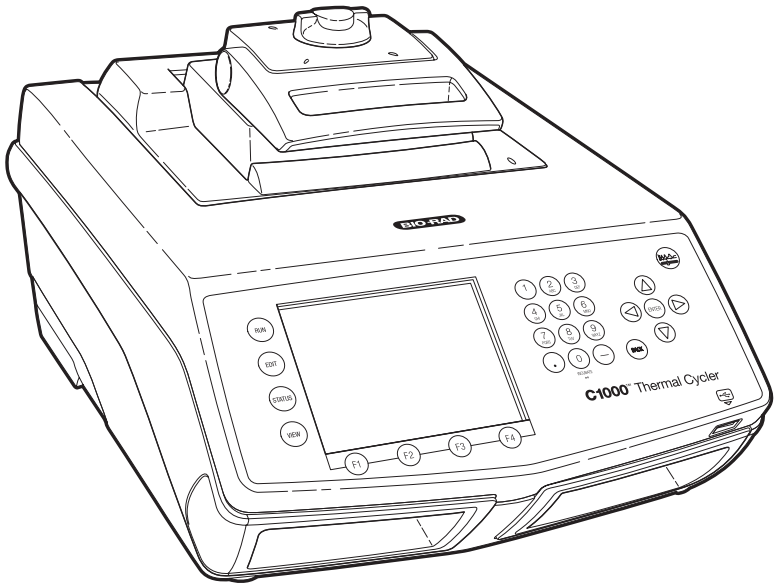
C1000™ Thermal Cycler

Instruction Manual

Catalog # 184-1000
185-1096
185-1048
185-1384



BIO-RAD

License Notice to Purchaser

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

This C1000 thermal cycler, when combined with a CFX™ detection module bearing a valid label license under U.S. Patent No. 6,814,934, constitutes a real time thermal cycler licensed under U.S. Patent No. 6,814,934 and corresponding claims in any Canadian counterpart patent thereof owned by Applera Corporation, for use solely in research and all applied fields except human and veterinary in vitro diagnostics, provided that the real time thermal cycler royalty fee that is applicable to said thermal cycler has been paid. No rights are conveyed expressly, by implication or estoppel to any patents on real time methods, including but not limited to 5' nuclease assays, or to any patent claiming a reagent or kit. For further information on purchasing license rights, contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, 94404, USA."

Safety and Regulatory Compliance

The C1000™ 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 1 to review the meaning of each safety warning label:

Table 1. Meaning of safety warning label.

	**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 C1000 thermal cycler:

Table 2. Instrument safety warning labels.

Icon	Meaning
	**Warning about risk of harm to body or equipment. Operating the C1000 instrument 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.**

Table 2. Instrument safety warning labels.

Icon	Meaning
	**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 Use Specifications and Compliance

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

Table 3. Safe use specifications.

Safe use requirements		Specifications
Input power	**Rated**	**100 - 240 Vac, 50 - 60 Hz.**
Fuses		**250 V, 10 A.**
Temperature	**Indoor use**	**Ambient temperature of 5°C to 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 No. 184-8000) with your 1000-series cyclers.**

Table of Contents

1 Introduction

Congratulations on purchasing the C1000™ thermal cycler, a core component in the Bio-Rad® 1000-series thermal cycling platform for running polymerase chain reaction (PCR) and real-time experiments. It combines powerful thermal performance with unsurpassed flexibility and expansion potential, making it ideal for both small and large laboratories.

This chapter provides an introduction to the C1000 thermal cycler and more, including:

The Bio-Rad 1000-series Platform

The 1000-series PCR instrumentation line is a flexible and modular platform that offers best-in-class performance and features in an easy-to-use reliable package. The 1000-series platform is designed for the way you want to work by seamlessly integrating your workflows with functions such as temperature optimization, vessel and sealer compatibility, programming options, online support, and USB connectivity.

Table 4. 1000-series products

Product	*Catalog Number*	*Item Description*
C1000 Thermal Cycler	184-1000	C1000 Thermal Cycler, Chassis only
C1000 Fast 96-well System	185-1096	C1000 Chassis + Fast 96-well Rxn Module
C1000 48/48-well System	185-1048	C1000 Chassis + Dual 48/48 Rxn Module
C1000 384-well System	185-1384	C1000 Chassis + 384-well Rxn Module
S1000 Thermal Cycler	184-2000	S1000 Thermal Cycler, Chassis Only
S1000 Fast 96-well System	185-2096	S1000 Chassis + Fast 96-well Rxn Module
S1000 48/48-well System	185-2048	S1000 Chassis + Dual 48/48 Rxn Module
S1000 384-well System	185-2384	S1000 Chassis + 384-well Rxn Module
96-well Fast Reaction Module	184-1096	96-well Fast Rxn Module For 1000-series

Table 4. 1000-series products

Product	*Catalog Number*	*Item Description*
48/48-well Fast Reaction Module	184-1048	Dual 48/48 Rxn Module for 1000-series
384-well Reaction Module	184-1384	384-well Rxn Module for 1000-series
CFX96 Real-Time System	185-5096	C1000 Chassis + CFX96 Rxn Module
CFX384 Real-Time System	185-5384	C1000 Chassis + CFX384 Rxn Module
CFX Software	184-5000	PC Software for use with Real Time PCR
CFX Software, Security Edition	184-5001	Single User License real time software
C1000 Manager Software	184-4000	Software for use with C1000 and attached S1000 cyclers

C1000 Thermal Cycler Performance and Features

The C1000 thermal cycler is the cornerstone of the 1000-series platform. It boasts powerful performance and intuitive usability as a standalone thermal cycler, with innovative features such as the Protocol Autowriter. Additionally, the flexible C1000 can be used with S1000 cyclers to expand throughput.

The C1000 Thermal Cycler Features:

- **Reliable Results with Precise Thermal Control: Sample block uniformity of ±0.4°C at 90°C after 10 seconds with an accuracy of ±0.2°C; adjustable lid temperature to further control reaction temperature and prevent evaporation.**
- **Flexible Vessel Format with interchangeable reaction modules: Simply change reaction modules when you need 48, 96, or 384-well blocks with the same thermal cycler base.**
- **Uniform and repeatable microplate sealing with adjustable lid force.**
- **Auto-generate Customized Protocols With the Protocol Autowriter Feature: Generate protocols based on user defined parameters.**
- **Easily Upgradable to 5-color, 6 channel Real-Time System: with the CFX real-time optical module.**
- **Reduced Protocol Run Times: The C1000 offers superior thermodynamics for shorter run times and expedited results.**
- **Encrypted Logs and Validation Reports: Simple GLP compliance on every run with automatically generated run logs, system logs, and validation reports. Optional administrative control over user access.**
- **Pre-installed, Customizable protocols to use as Templates: Speed protocol writing by customizing pre-loaded protocols.**
- **Ready for High-Throughput: Control up to 32 thermal cyclers. Use a bar code reader and temperature validation option to verify data flow and quality.**
- **Thermal Gradients: Optimize reaction performance using temperature gradients across sample blocks.**
- **Large Color Display: View and navigate through an intuitive user interface featuring large, full-color graphics and text.**

Bio-Rad Resources and References

Bio-Rad Laboratories provides many resources for scientists. The following web sites contain useful information for running PCR experiments:

- **Gene Expression Gateway (www.bio-rad.com/genomics/)**

  This site provides rich technical resources on a wide variety of methods and applications related to PCR and real-time PCR. This site also features tools, citations, technical support, and troubleshooting resources.

- **Life Science Research web site (discover.bio-rad.com)**

  This site includes links to technical notes, manuals, product information, and technical support.

Click the following links to download or request a copy of this manual or other Bio-Rad Laboratories literature:

- **Click the PDF icon to download a portable document format copy and open it using Adobe Acrobat Reader software (www.adobe.com).**
- **Click the folder icon and order a printed copy.**
- **Click the FAX icon to request a FAX copy.**
- **Phone your local Bio-Rad Laboratories office to request a printed copy. In the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the Literature option.**

Use the following resources to locate what you need:

Table 5. 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). Also find the nearest international office listed on the back of this manual.
Technical notes and literature	Go to the Gene Expression Gateway (www.bio-rad.com/genomics/) and locate the Search box in the upper, right corner of the web page. Type a search term in the box to find links to products, technical notes, and manuals.
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 technical support on the web, go to the Gene Expression Gateway (www.bio-rad.com/genomics). To find local technical support, 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.

Writing Conventions Used in This Manual

This manual uses the writing conventions shown in Table 6 to quickly provide relevant information.

Table 6. Manual Conventions

Convention	Meaning
TIP:	**Provides helpful instructions**
NOTE:	**Provides important information**
WARNING!	**Explains crucial information about a topic that may lead to injury, instrument damage, or data loss.**
`Screen` **message**	**Indicates the one or more words on the screen the user should select.**
`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` **by using the arrow keys or mouse. For example, "select** `NEW`**" means "Use the arrow keys or a mouse to highlight 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** `X` **key on the control panel. For example, "Press** `ENTER`**" means "Press the** `ENTER` **key on the control panel"**

2 Get Started

The C1000™ thermal cycler is easy to set up and run: insert a reaction module, plug in the thermal cycler, and begin a PCR experiment.

This chapter describes how to set up and operate the C1000 thermal cycler. Refer to the following sections for detailed instructions:

- Setting up the thermal cycler (below)
- Introducing the C1000 base and interchangeable reaction modules (page 6)
- Operating the control panel (page 8)
- Selecting files and folders from the file library (page 10)
- Logging in and out of the thermal cycler (page 13)
- Naming your thermal cycler (page 14)

Setting Up the C1000 Thermal Cycler

This section provides an overview of the steps needed to safely unpack and install the C1000 thermal cycler base and reaction module.

To install the thermal cycler and prepare to run PCR protocols, follow these steps:

1. Unpack the C1000 thermal cycler base and reaction module

   The C1000 base package includes the thermal cycler base, a power cord, 512MB USB drive, quick guide, and this manual. Keep all packaging to safely move this instrument. Place the thermal cycler base in the desired location.

   NOTE: A thermal cycler requires sufficient cool air flow to run precisely. To check for sufficient air flow before running the instrument, see“Maintaining Sufficient air flow” on page 95.

   Keep all packaging to safely move this instrument. Place the thermal cycler base in a suitable location for running PCR (see “Instrument Operating Specifications” on page 95).

2. Insert a reaction module into the thermal cycler base.

   The reaction module slips into the base and locks in place for operation. For detailed instructions about loading a reaction module, see “Inserting and Removing a Reaction Module” on page 18.

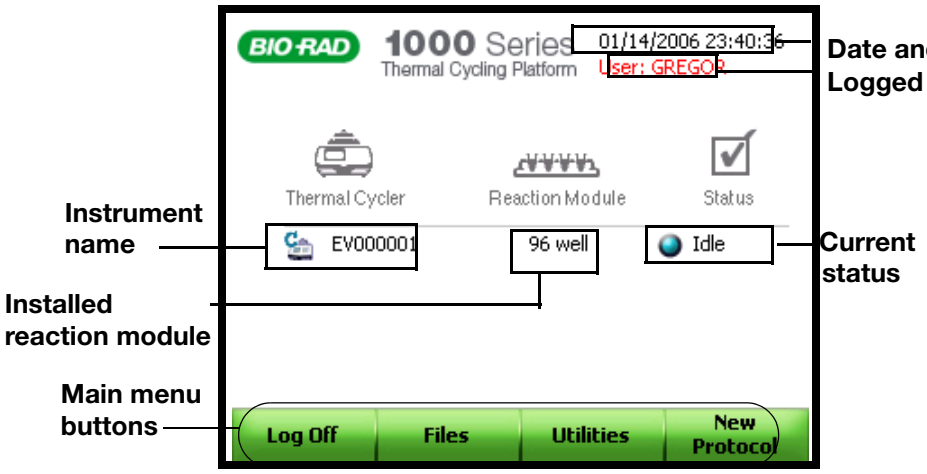
3. **Plug in the power cord and turn on the thermal cycler.**

   Use the supplied power cord to plug the thermal cycler base into an appropriate electrical outlet ("Instrument Operating Specifications" on page 95). Use the power switch at the back of the base to turn on the power (page 8).

   NOTE: Before operating the thermal cycler, be sure to read the safety specifications ("Safety and Regulatory Compliance" on page 3) and operating requirements ("Appendix B: Operating Specifications" on page 95).

   When it starts, the thermal cycler runs a self-test to verify proper functions, and then displays the main menu. Use the main menu to begin operating the thermal cycler. This window provides access to all thermal cycler operations, displays the status of the reaction module and the thermal cycler name.

   For example, the main window displays the date and time, name of logged in user (`GREGOR`), instrument name (`EV000001`), reaction module (`96 well`), current status (`Idle`), and `Main Menu` buttons:



For details about using the control panel and main menu, see page 8. For instructions about running a PCR protocol, see "Run Protocols" on page 33.

> **TIP: To get started quickly, the C1000 thermal cycler comes with six groups of preinstalled PCR protocols ("Appendix A: Preinstalled Protocols" on page 109).**

This 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. For more information about contacting your local Bio-Rad Laboratories office, see "Bio-Rad Resources and References" on page 3.

C1000 Thermal Cycler Overview

The following sections describe important parts of the thermal cycler and reaction module:

- **Front view of the base without reaction module (page 7)**
- **Top view of the base with reaction module (page 7)**

- Back panel with data ports (page 8)

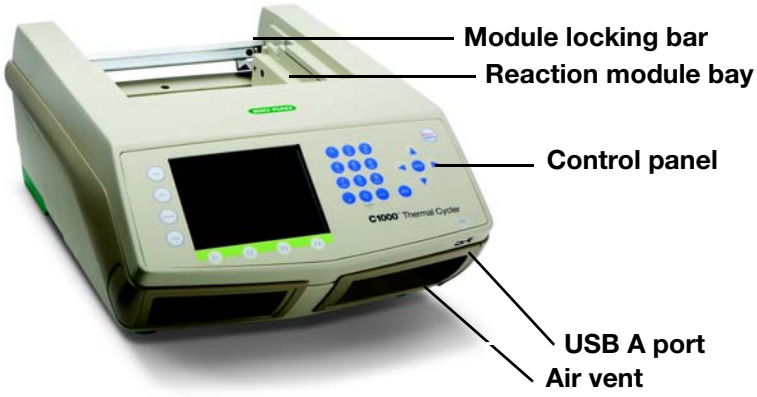
Table 7. 1000-series reaction module size and limits

Number of wells	Number of blocks	Recommended Sample Volume (Upper Limit)
Dual-48	2	10 – 50 uL (50 uL limit)
96	1	10 – 50 uL (50 uL limit)
384	1	3 – 30 uL
CFX 96 Real-Time	1	10 – 50 uL (50 uL limit)
CFX 384 Real-Time	1	3 – 30 uL

Front View Without Reaction Module

The front of the thermal cycler base includes a control panel and reaction module bay. The control panel provides access to all the functions needed to create and run PCR protocols.

- Reaction module bay: Holds the inserted reaction module
- Reaction module locking bar: Locks reaction module in place
- Control panel: Provides access to all the functions needed to create and run PCR protocols (page 9)
- USB A port: Connects to a USB drive, computer mouse, or other USB devices (page 10)
- Air vents: Allows the thermal cycler to cool and heat quickly

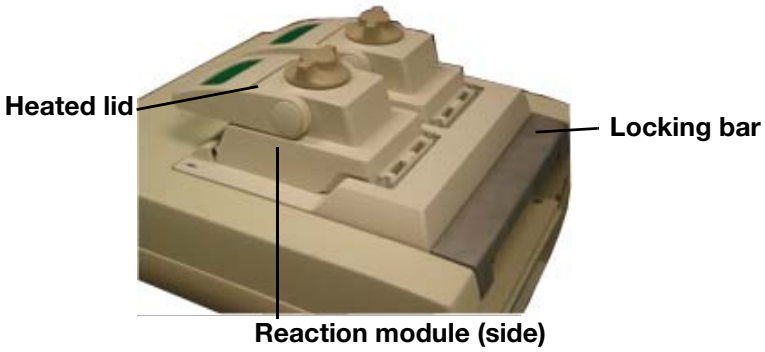


Top View With Reaction Module

This top view shows the back of a reaction module docked in the bay of the thermal cycler base. The following shows features on the back of the reaction module and thermal cycler base, including these parts:

- Heated lid: Maintains lid temperature and force
- Status LED: Lights when reaction module is running
- Locking bar: Locks reaction module into the thermal cycler base

For more information about reaction modules, see "Reaction Modules" on page 17.

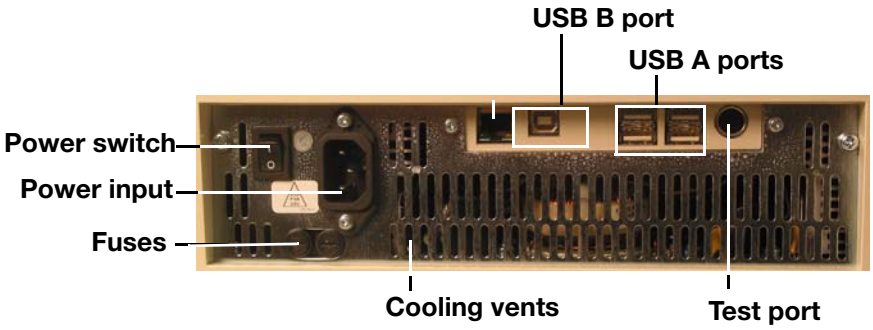


Back Panel of Thermal Cycler Base

The back panel includes data ports that transfer data to and from the thermal cycler. The following image shows the ports on the back panel.

- **USB B port: To connect the C1000 thermal cycler to a computer.**
- **USB A ports: To connect the C1000 to one or more S1000 thermal cyclers (see "Control an S1000 With the C1000" on page 105).**

  These ports can also connect to any USB peripheral, the most common are USB mouse, keyboard, drive, or bar code reader.
- **Test port: For service testing only.**

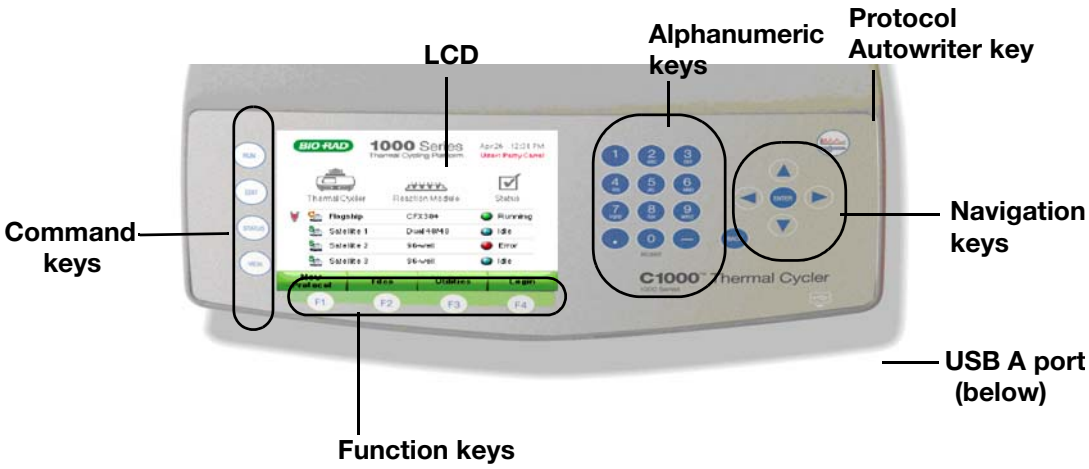


Control Panel and Main Menu

The control panel on the C1000 thermal cycler provides access to all the functions needed to run the thermal cycler. The control panel includes the following three components:

- **Liquid Crystal Display (LCD): Displays the main menu and other screens.**
- **Command, Function, Alphanumeric keys and the Protocol Autowriter button: Enter commands with these keys.**
- **USB A Port: Connect a USB drive, mouse, keyboard, or bar code reader(page 10).**

The main screen is displayed after booting is complete. When the instrument starts up, it goes through 2 other screens: the black booting screen and the self-test screen. This shows the components of the control panel:

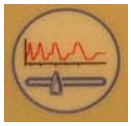


Functions of Control Panel Keys

The control panel contains five sets of keys with the functions listed below:)
Table 8. Functions of keys on control panel

Key	Function	Additional notes
COMMAND KEYS **Four keys that instruct the thermal cycler to begin a process**		
RUN	**Select and run a protocol**	
EDIT	**Select and change a protocol**	
STATUS	**View the status of one or more running protocols on three screens**	**Pressing this key cycles through the main menu, the protocol screen, and the block status list**
VIEW	**Switch between graphic and text view of a protocol; also toggles through screens in Status.**	
FUNCTION KEYS		
F1, F2, F3, or F4	**Activate the four buttons options at the bottom of each screen using the function keys**	
ALPHANUMERIC KEYS		
1 **through** 9	**Enter numbers or letters of the alphabet**	**Press each key multiple times to switch between number and associated letters**
0, INCUBATE	**Insert a zero, infinity, or start instant incubation**	
PROTOCOL AUTOWRITER		

Table 8. Functions of keys on control panel

Key	Function	Additional notes
	**Launch the Protocol Autowriter ("Protocol Autowriter Overview" on page 23)**	
NAVIGATION KEYS		
`RIGHT` **arrow**	**Move cursor to the right**	**Expands folders in the file library**
`LEFT` **arrow**	**Move cursor to the left**	**Collapses folders in the file library**
`UP` **arrow**	**Move cursor up**	
`DOWN` **arrow**	**Move cursor down**	
`ENTER`	**Confirm selection**	
`BACK`	**Delete a letter, number, or word; also goes back to previous screens**	

Main Menu

The main menu is displayed on the main screen, which provides links to all C1000 functions, and displays any attached S1000 thermal cyclers.

> **NOTE: The buttons' names and functions change with each screen.**

To initiate the functions in the main menu, press the associated function keys (`F1` through `F4`) or, click the button using a computer mouse, on the control panel:

- `New Protocol` **(`F4`): Press the key to create a new protocol ("Opening a New Protocol or an Existing Protocol" on page 47).**
- `Utilities` **(`F3`): Press this key to open the `Utilities` menu (page 101).**
- `Files` **(`F2`): Press the key to view the files and folders in the file library ("Protocol File Content" on page 79).**
- `Log In` **(`F1`): Press this key to log in to the C1000 thermal cycler. Once you log in the button name changes to `Log Off`.**

USB A Port

The C1000 has one USB A port below the control panel. This front USB A port provides an easy way to transfer data using a USB drive or to connect a mouse.

> **TIP: Connect a USB drive, computer mouse, or a S1000 thermal cycler to any USB A port on the front or the back of the C1000 ("Using Data Ports" on page 104).**

The File Library

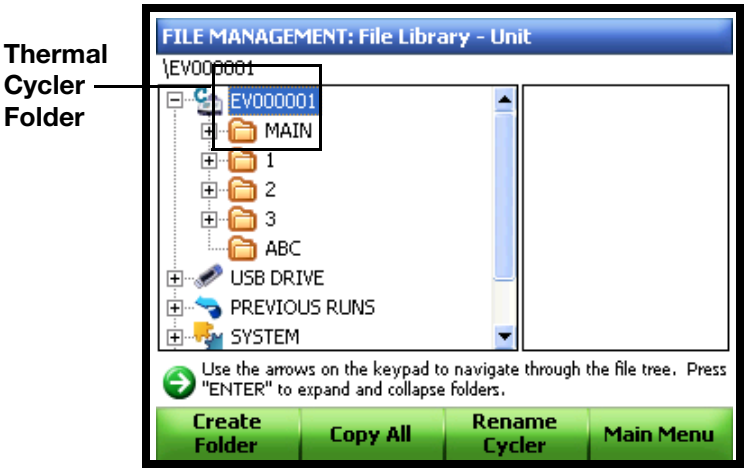
The C1000 file library provides easy access to protocol files, folders, and system logs.

Many functions begin with choosing a protocol from the file library. For example, before running or editing a protocol, select the desired protocol file in the file library:

- **Press the `RUN` key to run a protocol**
- **Press the `EDIT` key to edit a protocol**

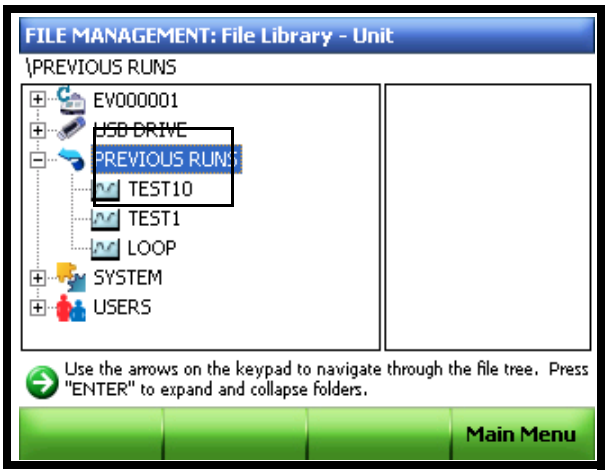
To open the file library, select `Files` in the main menu. The file library provides access to the following folders:

- **Thermal cycler protocol folder: Contains protocols stored on the thermal cycler, including the `MAIN` folder that stores the preinstalled protocols. This folder is named after the thermal cycler's serial number unless the thermal cycler has been renamed:**

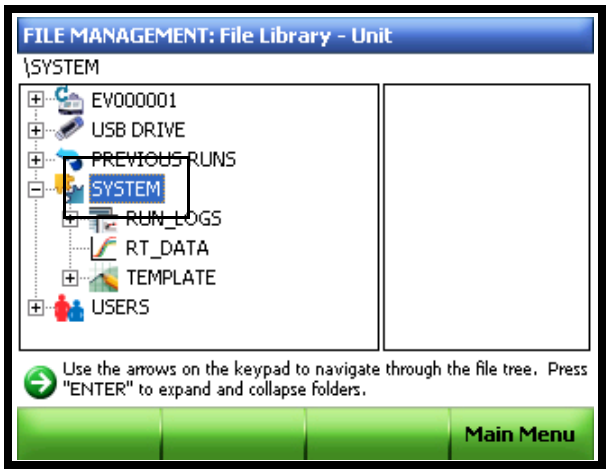


NOTE: When you connect an S1000 thermal cycler to the C1000 thermal cycler, the attached thermal cycler displays a folder. That folder is named with the thermal cycler name. The default thermal cycler name is the serial number of the base.

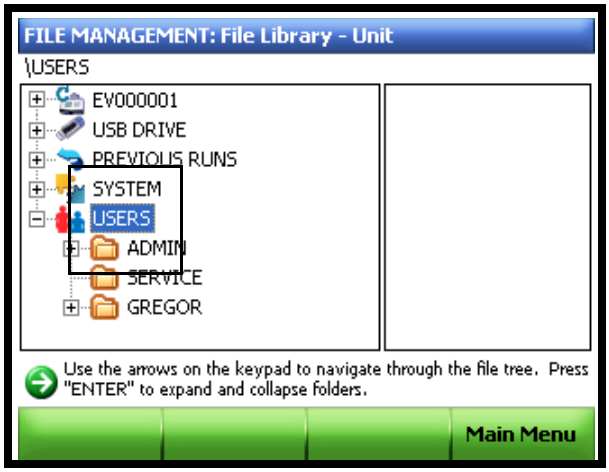
- `PREVIOUS RUNS` **folder: Contains a list of all previously run protocols.**



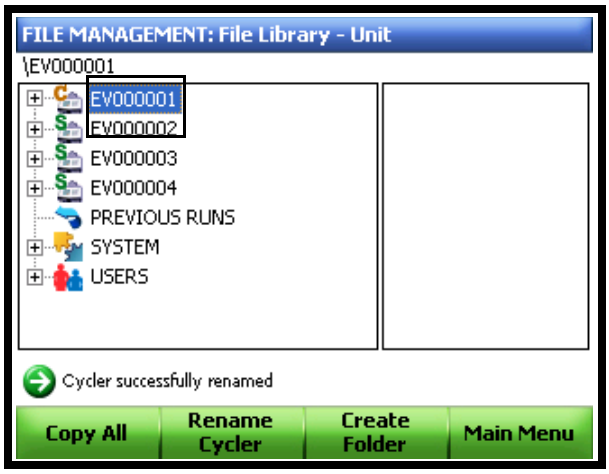
- SYSTEM **folder: Contains run logs, real-time data, and templates:**



- USERS **folder: Contains user-created folders for personal protocol storage. The User folders can be accessed by everyone but only a logged in user can edit, delete or save into these folders ("Logging In and Logging Off the C1000 Thermal Cycler" on page 13).**



- **Attached folders (optional): Contains the data on attached USB drives, or S1000 thermal cyclers (page 105). In this example, three connected S1000 thermal cyclers are displayed on the C1000 thermal cycler:**



Logging In and Logging Off the C1000 Thermal Cycler

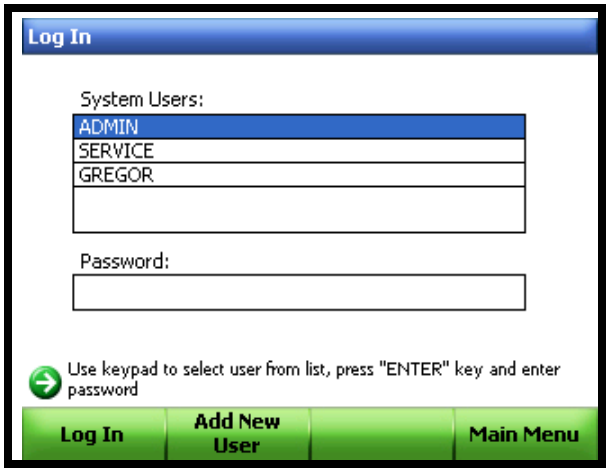
Logging in is optional; you will still be able to run, edit, and create folders and protocols. When a user registers, a corresponding folder under `USERS` **will be created automatically. Only a logged in user can save, edit or delete these protocols.**

Once logged in, a user has the following privileges:

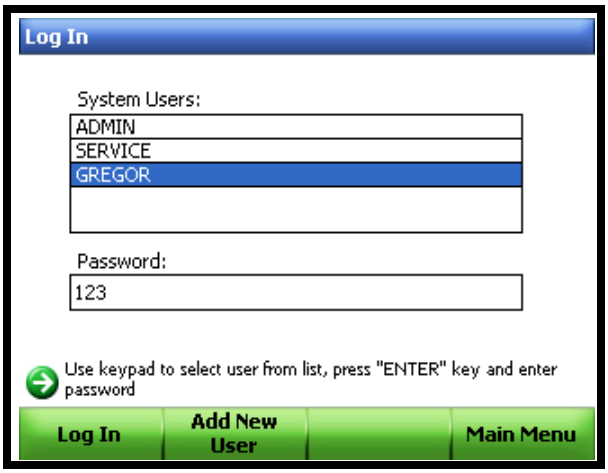
- **Run a protocol in the** `MAIN` **folder or a user folder**
- **Create and edit protocols in the corresponding user folder**
- **Copy any protocol and save it to a user folder**
- **Copy a protocol and store it in a user folder with a new name**

Use the main menu to log in and out by following these steps:

1. **Select** `Log In` **in the main menu to open the Log In screen:**



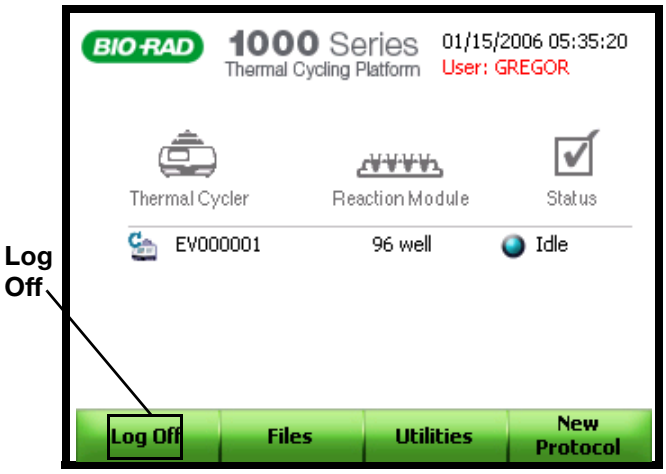
2. **Select your user name (using the `UP` & `DOWN` arrows), enter your password, and then press `F1` or click `Log In`:**



NOTE: A password can contain letters and numbers.

3. **Select `Main Menu` to return to the main menu.**

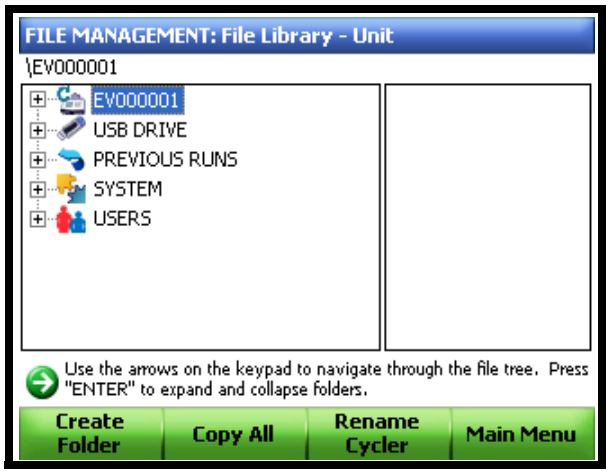
4. **To log off the thermal cycler, press `F1` or select `Log Off` in the main menu:**



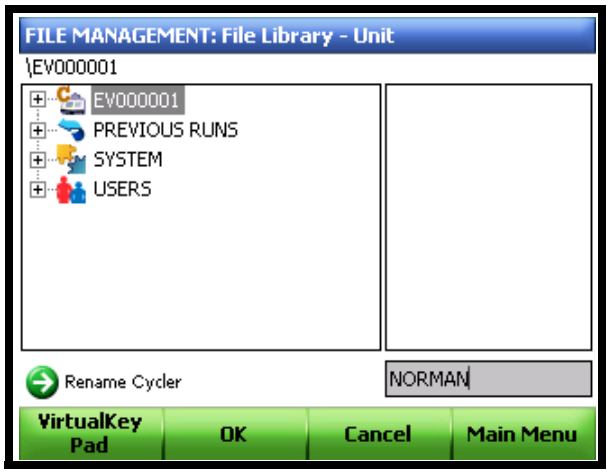
Renaming the C1000 Thermal Cycler

Each C1000 thermal cycler is initially named using the serial number of the thermal cycler base. A logged in Administrator can rename any thermal cycler for easy identification. To rename a C1000 thermal cycler, follow these steps:

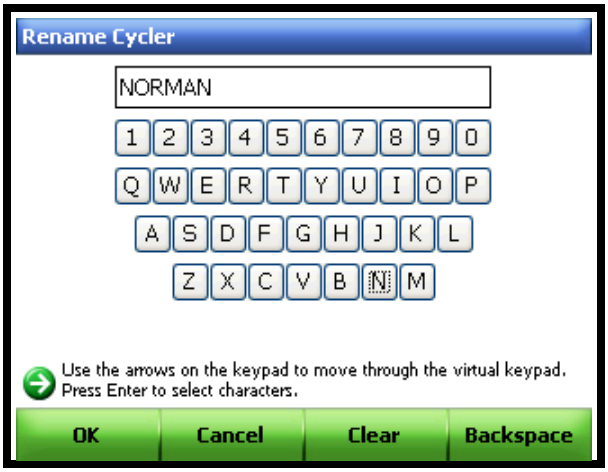
1. **Select** `Files` **to open the file library, and then select** `Rename Cycler`



2. **Type a new name for the thermal cycler**



Alternatively, open the `Virtual Keypad` **to type a name using the onboard keyboard. Use the arrow keys to highlight letters and numbers and press** `ENTER` **to select them:.**



3. **Select** `OK` **to accept the new cycler name**

3 Reaction Modules

The C1000™ thermal cycler runs interchangeable reaction modules to enable a user to change block formats easily. This chapter describes how to set up and operate the reaction modules. Refer to the following sections for detailed instructions:

- Overview of the reaction modules (below)
- Inserting a reaction module (page 18)
- Operating the lid (page 20)

Interchangeable Reaction Modules

The C1000 thermal cycler is compatible with any 1000-series reaction module. 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. The reaction modules come in three block sizes:

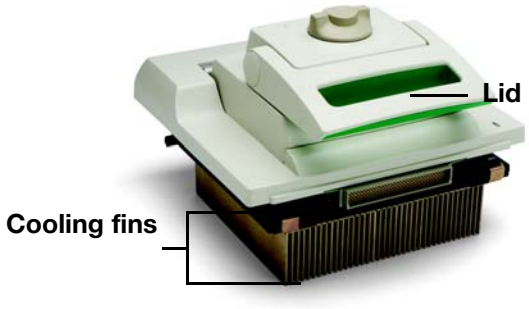
Table 9. 1000-series reaction module size and limits

Number of wells	Number of blocks	Recommended Sample Volume (Upper Limit)
Dual-48	2	10 – 50 uL (50 uL limit)
96	1	10 – 50 uL (50 uL limit)
384	1	3 – 30 uL
CFX 96 Real-Time	1	10 – 50 uL (50 uL limit)
CFX 384 Real-Time	1	3 – 30 uL

The following shows a 96-well reaction module with the following features:

- Cooling fins: Fins allow fast heating and cooling.

- **Lid: Adjustable, heated lid:**

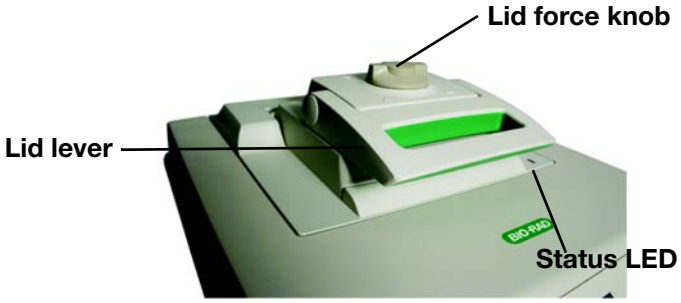


The inside of a reaction module includes the features below:

- **Heated inner lid: Raises and lowers heat to prevent condensation and evaporation**
- **Sample block: Holds reaction vessels, including tubes and microplate:**

The top of a reaction module lid includes the following features:

- **Lid lever: Use this lever to open and close the lid.**
- **Lid force knob: Adjust this knob to set lid force and seal the reaction.**
- **Status LED: This light turns on to indicate that the block is selected or running.**



Inserting and Removing a Reaction Module

Insert the reaction module and lock it into the thermal cycler base for safe operation. This section includes instructions for inserting and removing the reaction module.

Inserting a Reaction Module

Inserting a reaction module takes a few seconds. To insert a reaction module follow these steps:

1. **Always turn off the thermal cycler base before inserting or removing a reaction module**
2. **Align the reaction module in the bay**

   Lift the module into the reaction module bay with the lid lever pointing to the front. Leave about 1-2 cm of space in front of the module.

3. **Lock the reaction module in place**

   Pull the locking bar up to lock the module in place. Notice that there is no space at the front of the module when it is locked into the base.

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

Removing a Reaction Module

Follow these steps to remove a reaction module from the base:

1. **Always turn off the thermal cycler base before removing a reaction module**
2. **Unlock the reaction module**

   Push the locking bar down to release the module:

3. **Lift the reaction module out of the bay**

   Before lifting the reaction module, make sure that the cooling fins are not hot, then carefully lift the reaction module.

   WARNING! Cooling fins might be hot immediately after running a protocol or incubation.

   Store the reaction module on a clean, flat surface where it cannot get bumped, scraped, or dropped:

Cooling fins

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

Operating the Adjustable Lid

The reaction module lid applies both heat and force on the reaction vessel lids (caps or tape) with the inner lid. This heat and force helps produce consistent and successful reactions:

- **Applying a force to the reaction vessel lid seals the reaction to prevent evaporation**
- **Heating over the reaction vessel lid prevents condensation**

The following sections describe how to open and close the lid, and how to adjust the lid force.

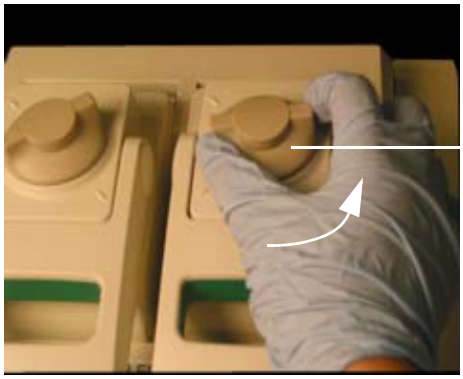
WARNING! After a run, the heated lid can remain hot. Use caution when opening and closing the lid. Be sure to avoid touching the sealing plate or block unless it has cooled.

Opening the Lid

To open the lid, follow these steps:

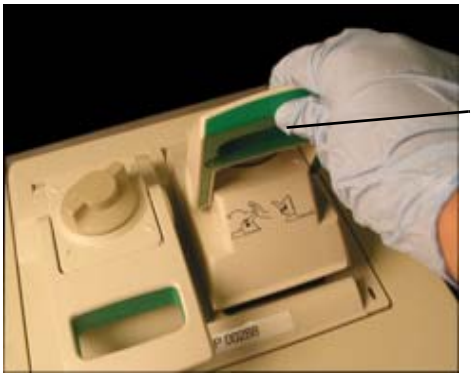
1. **Turn the lid force knob to release the lid**

   Twist the lid force knob in a counterclockwise direction to release the lid:



2. **Lift the lever and push back**

   To open the lid, push the lid lever back and lift:



3. **Open lid completely**

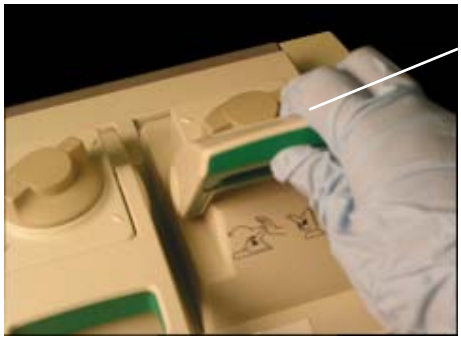
   Continue lifting the lid lever until the reaction module lid stays open without assistance.

Closing the Lid and Setting Lid Force

When you close the lid, select the lid force by turning the lid force knob. To close the lid, follow these steps:

1. **Close the lid**

   Push the lid lever down and make sure the front of the lid is secured beneath the housing, and lock it in place:



2. **Adjust the lid force**

   Turn the lid force knob to adjust the lid force:
   - **To increase the lid force, turn the knob 1/4 turn clockwise (to the right).**
   - **To decrease the lid force, turn the knob 1/4 turn counterclockwise (to the left).**

   Adjust the lid force to a similar setting each time by turning it to the same position. Notice that position marks on the lid indicate 1/4 turns.

Loading Sample Vessels into the Block

When using a small number of tubes, they should all be placed in the center of the block, to ensure uniform thermal cycling of all samples. Also, load at least one empty tube in each corner of the block, to ensure that the lid exerts even pressure on the sample tubes.

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

- **Ensure that the block is clean before loading samples**
- **Firmly press individual tubes or the microplate into the block wells**

TIP: When using only one or a few tubes, use the Tube Tray (Catalog Number 184-7000) to provide extra support

4 Protocol Autowriter

The C1000™ thermal cycler is equipped with the Protocol Autowriter. Autowriter provides an alternative programming method based on the characteristics of the amplicon and the primers as well as the desired reaction speed.

Read this chapter to learn more about the following topics:

- **Overview of the Protocol Autowriter (below)**
- **Writing a protocol with the Protocol Autowriter (page 24)**
- **Background information on the Protocol Autowriter and T_a Calculator (page 29 and page 30, respectively)**

Protocol Autowriter Overview

The Protocol Autowriter uses information about your reaction to automatically write a protocol. Enter the following information about your PCR experiment:

- **Primers: Enter the reaction annealing temperature (`Ta`) for the primers being used. If you are unsure of the annealing temperature, enter the primer sequence in the `Ta Calculator` and the Autowriter will calculate the T_a.**

  Note: The T_a is adjusted from the primer T_m information, based on the selected enzyme and the protocol speed selected.
- **Amplicon length: Enter the expected length of the PCR product.**
- **Enzyme type: Enter the DNA polymerase enzyme (iTaq™, iProof™, or `Other`). If you use an enzyme other than iTaq or iProof polymerase, enter additional information including the hot-start activation time and the final extension time.**

1. **Run speed: Select a reaction speed (Standard, Fast, Ultrafast).The Protocol Autowriter optimizes the protocol depending on the speed setting that you choose. For example, a typical PCR protocol includes the following three sets of steps with a total run time of 1.5 to 2.0 hours:**
2. **Initial template denaturation and enzyme activation (95°C for 3–10 min.).**
3. **Cycles of these three temperature steps (30 to 40 cycles):**
   - **Denaturation of template (94–95°C for 15–30 sec)**
   - **Annealing of primers (Anneal for 15–30 sec)**
   - **Extension of product (72°C for 15–60 sec)**
4. **Final extension (72°C for 10 min.).**

The total run time is determined by the number of steps and cycles, the incubation time at each step, and the time it takes to reach uniformity at the target temperature.

To reduce the overall run time, the Protocol Autowriter makes one or more of the following changes:

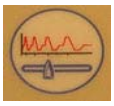
- **Reduce the total number of protocol steps (for high T_m primers, the annealing and extension steps can be combined into one step)**
- **Reduce the number of cycles**
- **Reduce the hold time in each temperature step**
- **Reduce the ramp time between steps by reducing the temperature change from one step to the next**

  For example, the Protocol Autowriter might make the following modifications to shorten a protocol:

  - **Change the initial template denaturation and enzyme activation step from 95°C for 3 minutes to 98°C for 30 seconds**
  - **Change the denaturation step in each cycle from 95°C for 30 seconds to 92°C for 1 second**
  - **Combine annealing and extension into a single step at 70°C for 20 seconds**

Creating a Protocol With the Protocol Autowriter

Follow these steps to run the Protocol Autowriter and create a new protocol:

1. **Press the Protocol Autowriter key** 

   This launches the Protocol Autowriter.

2. **Enter the Annealing Temperature in °C and Amplicon Length in base pairs**

   If you do not know an appropriate annealing temperature for your primers, select the T_a Calc button (F1), enter the primer sequences using the A, G, C, & T buttons then press Calculate. The Autowriter will calculate the primers' T_ms and an appropriate annealing temperature (T_a). Press OK (F4) to return to the main Autowriter screen.

   NOTE: The T_a Calculator generates an annealing temperature for Standard speed with iTaq polymerase. Different enzyme speed settings will automatically adjust T_a.

   For more information on how the T_a calculator works, refer to "Ta Calculator - Background" on page 30.

3. **Select the enzyme to be used by pressing the UP and DOWN arrow keys, then press ENTER**

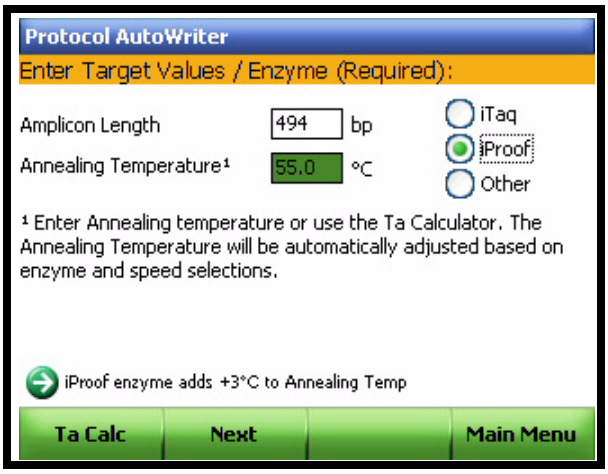
4. **Finally, select Next to continue to the Run Time screen**

   Use the RIGHT and LEFT arrow keys to select the desired reaction speed on the slider bar.

Examples of using the Autowriter:

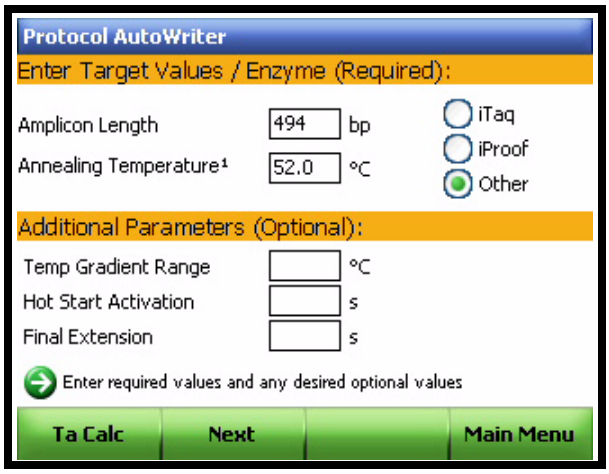
A. Annealing Temperature is known

- **The amplicon under consideration has been successfully amplified with an annealing temperature of 52°C; it is 494 base pairs long**
- **In the first Protocol Autowriter screen ["Enter Target Values / Enzyme (required)"]**
  - **Enter `494` in the Amplicon Length box, then `DOWN` arrow or `ENTER` to go to the Annealing Temperature box**
  - **Enter `52` in the Annealing Temperature box; then `RIGHT` arrow or `ENTER` to go to the Enzyme options.**
  - **The default selection is "iTaq". If "iProof" is to be used, `DOWN` arrow to iProof**

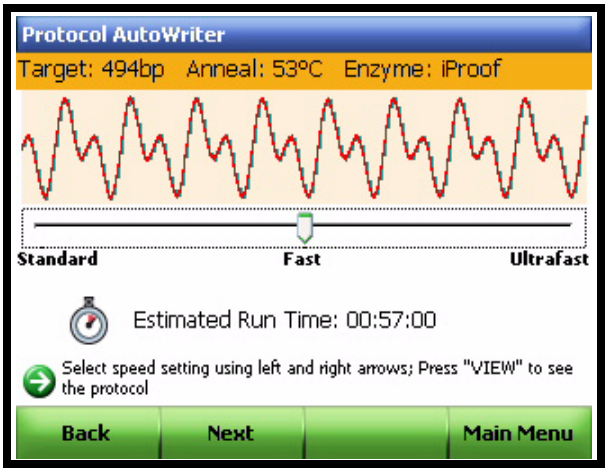


NOTE: 3°C are automatically added to the Annealing Temperature if iProof is selected.

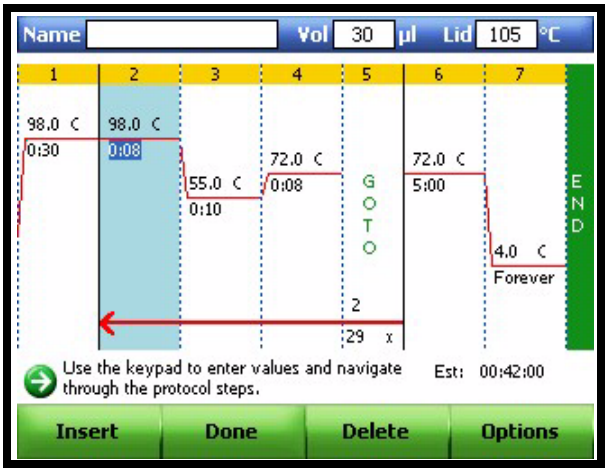
  - **If "Other" is selected, additional options are presented such as a Hot Start time**



- **Press** `F2` **(**`Next`**) to go to the reaction speed screen. The Target size, the Annealing Temperature and the selected enzyme are listed across the top of the screen.**



- **Select the desired speed using the** `RIGHT` **and** `LEFT` **arrow keys; note the change in the Annealing Temperature and in the estimated total run time as the speed is changed. The annealing temperature used in the program will be 4°C below the primer average T_m for the Standard Speed, 2°C below the primer T_m average for Fast, and the un-adjusted primer T_m average will be used for the UltraFast speed. These times are displayed on this speed selection screen as well as on later program display screens**
- **Select** `F2` **(**`Next`**)**
- **The Autowriter protocol is now displayed in a graphic format**

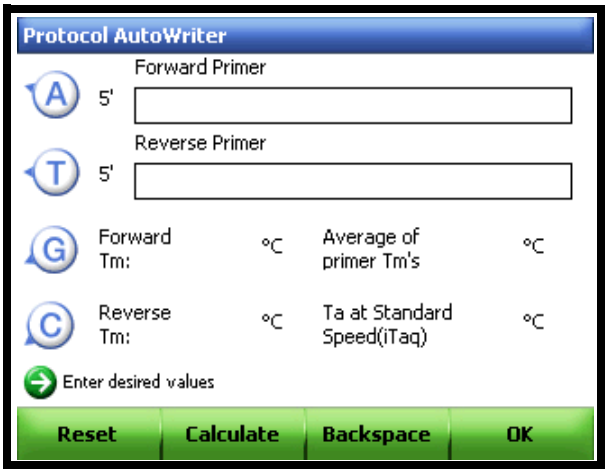


- **Pressing the `VIEW` button will display the program in a textual format**

- **Press `VIEW` again to return to the graphic display. All aspects of the program can be edited from either format view by navigating to the desired step with the arrow keys then using the various action keys**

B. Annealing temperature not known

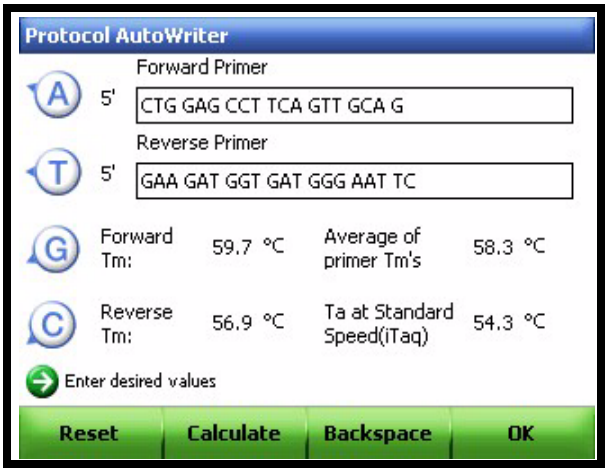
- **To have the Autowriter determine the annealing temperature, press `Ta Calculator` key (`Ta Calc, F1`) from the main Autowriter screen**



- **Enter the primer sequences in the sequence boxes using the four buttons along the left side of the screen corresponding to A, T, G & C. For example1:**
- **Forward primer: CTG GAG CCT TCA GTT GCA G**
- **Reverse primer: GAA GAT GGT GAT GGG ATT TC**

  To correct a wrong base entry: press `BACK` or `Backspace` (`F3`) to erase the base immediately to the left of the cursor. If necessary, use the `LEFT` and `RIGHT` arrow keys to position the cursor within the primer sequence. `RESET` will clear the entire sequence.

- **Then press** `F2 CALCULATE`**. The T_m of each primer, the average of the T_ms and the Annealing Temperature (T_a) are all calculated and displayed on the screen**



- **Note that if the primer T_ms are greater than 4°C apart, the Autowriter uses the lower primer T_m +2°C as a basis for calculating the T_a, which can be further modified by enzyme and reaction speed selections**

  NOTE: The T_a Calculator generates an annealing temperature for Standard speed with iTaq polymerase. Different enzyme speed settings will automatically adjust T_a.
- **Press** `OK` **to go to the main Autowriter screen**
- **The Annealing Temperature (T_a) box is automatically filled in with the T_a from the previous screen**
- **Proceed as in example A, above.**

  The amplicon used in the example is a from a glyceraldehyde phosphate dehydrogenase sequence in the human genome (GenBank acc# J04038)

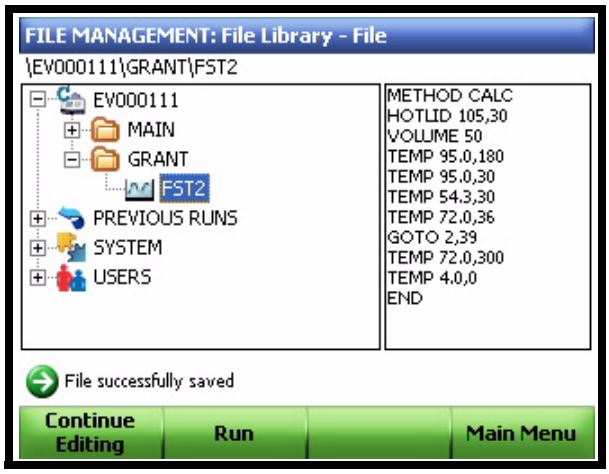
5. **Review the protocol and add options**

   Review the new protocol in the graphic view. To change to text-based editing press the `VIEW` **key. To add options to a selected protocol step, press the** `Options` **button (**`F4`**) or press** `Delete` **(**`F3`**) to delete the highlighted step, or** `Insert` **(**`F1`**) to insert a new step after the highlighted step (page 48)**

6. **Save the new protocol**

   Select `Done` **to save the protocol, then select the folder where you want to save the protocol by pressing the arrow keys. To collapse a folder, press the** `LEFT` **arrow key. To open a folder press the** `RIGHT` **arrow key. You can also use** `Create Folder` **for a new folder. Finally, select** `Save` **to store the new protocol in the selected folder.**

In this example, the protocol is named FST2 and saved in the GRANT folder:



7. **Run the new protocol, or return to the main menu**

   Select `RUN` **to run the protocol, or select** `Main Menu` **to return to the main menu.**

   NOTE: If you decide to make further changes to this protocol, select the Continue Editing button.

Protocol Autowriter - Background

The protocol autowriter software uses standard PCR guidelines that automatically generate cycling protocols with initial template denaturation and enzyme activation, followed by cycles of denaturation, annealing and extension, then final extension steps. Protocols are based on user input parameters of target amplicon length, enzyme type and annealing temperature or primer sequences.

Protocols generated by the Protocol Autowriter at various speed settings (Standard, Fast and Ultra-fast) may result in different product yields. This is because to generate these protocols, the Protocol Autowriter may adjust the annealing temperature, reduce the total number of protocol steps, reduce the number of GOTO repeats, shorten hold times, or reduce the temperature differentials between steps.

The Protocol Autowriter uses established PCR standards that references data tables to produce the final suggested protocols. All protocols are either a standard 2-step or 3-step with a final extension step.

The steps and range of values generated by the Protocol Autowriter are as follows:

1. **Initial Hot Start Activation/Denaturation Step - between 95-98°C for 30 or 180 sec, depending on enzyme type and speed setting.**

2. **Denaturation Step - 95 or 98°C for between 5 - 30 sec, depending on enzyme type and speed setting.**

3. **Annealing Step - The Protocol Autowriter uses the either the Primer T_a at "standard" speed as calculated by the T_a calculator or a user input value.**
   - **The annealing time ranges from 10 - 30 seconds depending on the speed setting. Two-step protocols combine Annealing and extension steps.**
   - **The annealing temperature (T_a) is calculated based on primer characteristics and selected reaction speed. See "T_a Calculator**

Background" for more information on how the T_a calculator functions. If the iProof enzyme is selected, 3 degrees are added to the T_a.

4. **Extension Step (3-Step Protocols Only) are all at 72°C with extension times based on calculating the times from set times per kbp (e.g. 60sec/kbp) of the largest amplicon in a size bin, often with some modifications at the smaller amplicon sizes.**
5. **Number of Repeats- Between 25 and 40 depending on speed selected**
6. **Final Extension Step- 72°C between 1 - 5 minutes depending on speed settings.**

T_a Calculator - Background

The T_a Calculator calculates the T_ms for each primer as well as the T_a for the protocol at "standard" speed.

The T_a for the protocol is based on the average of the primer T_m, with the following applied:

- **If the difference between the primer T_ms is > 4, the T_a = (lower of the two primer T_ms +2) - 4°C.**
- **If the difference between the T_ms is < or = 4, the T_a = (average of the primer T_ms) - 4°C.**

For each primer, the T_a Calculator uses the base pair counting method for sequences of 14 pairs or less.

- **T_m = ((w*A + x*T) * 2) + ((y*G + z*C) * 4)**

  where w,x,y,z are the number of the bases A,T,G,C in the sequence, respectively.

For sequences longer than 14 bp, the nearest neighbor method is used:

In the nearest neighbor method, the melting temperature calculations are based on the thermodynamic relationship between entropy enthalpy, free energy and temperature, where:

$\Delta H = \Delta G + T^* \Delta S$ where

ΔH = Enthalpy value, Cal/Mole*K

T = temperature, Kelvin.

ΔS = Entropy value, Cal/Mole*K

ΔG = Gibbs free energy in Cal/Mole*K

The change in entropy (order or a measure of the randomness of the oligonucleotide) and enthalpy (heat released or absorbed by the oligonucleotide) are directly calculated by summing the values for nucleotide pairs obtained by Breslauer et al., Proc. Nat. Acad. Sci. 83, 3746-50, 1986.

The relationship between the free energy and the concentration of reactants and products at equilibrium is given by:

$\Delta G = R*T*\ln ((DNA * Primer) / (DNA + Primer))$

where R is the Gas Constant(1.986 Cal/Mole*K)

Substituting G in the two equations and solving for T gives

T = ∆H / (?S + R*Ln((DNA * Primer) / (DNA + Primer)))

Assumes the concentration of DNA and the concentration of the DNA-primer complex are equal.

It has been determined empirically that there is a 5 (3.4 by Sugimoto et al.) kcal free energy change during the transition from single stranded to B-form DNA.

This is presumably a helix initiation energy. Finally, adding an adjustment for salt gives the equation that the T_a Calculator uses:

T = (∆H - 5(KCal/K*Mole)) / (∆S + (R * ln(1 / (primer)))) + 16.6Log10(SaltMolarity)

No adjustment constant for salt concentration is needed, since the various parameters were determined at 1 Molar NaCl, and the log of 1 is zero.

ASSUMPTIONS:

The thermodynamic calculations assume that the annealing occurs at pH 7.0. The melting temperature (T_m) calculations assume the sequences are not symmetric and contain at least one G or C.

The oligonucleotide sequence should be at least 14 bases long to give reasonable T_ms. Less than 14 bases uses the base pair counting method

Breslauer interaction constants:

Breslauer, et al

INTERACTION		delta(H)	delta(S)	delta(G)
AA	TT	9.1	24	1.9
AT	TA	8.6	23.9	1.5
AC	TG	6.5	17.3	1.3
AG	TC	7.8	20.8	1.6
TA	AT	6	16.9	0.9
TT	AA	9.1	24	1.9
TC	AG	5.6	13.5	1.6
TG	AC	5.8	12.9	1.9
CA	GT	5.8	12.9	1.9
CT	GA	7.8	20.8	1.6
CC	GG	11	26.6	3.1
CG	GC	11.9	27.8	3.6
GA	CT	5.6	13.5	1.6
GT	CA	6.5	17.3	1.3
GC	CG	11.1	26.7	3.1
GG	CC	11	26.6	3.1

5 Run Protocols

The C1000™ thermal cycler runs protocols on one or more reaction module blocks. Before running the protocol, you have the option to edit the protocol (page 48). For your convenience, the C1000 thermal cycler includes a set of preinstalled, standard protocols that you can run (page 109).

For instructions on running protocols, read the sections in this chapter on how to:

- **Run a protocol with the** RUN **key (below)**
- **Monitor a protocol that is running with the** STATUS **and** VIEW **keys (page 37)**
- **Pause and resume a protocol that is running with the** Pause **and** Resume **buttons (page 40)**
- **Skip steps while running a protocol with the** Skip Step **button (page 41)**
- **Stop a running protocol with the** CANCEL **key (page 42)**
- **Press the** INCUBATE **key to instantly incubate samples at a single temperature (page 44)**

Running a Protocol With the RUN Key

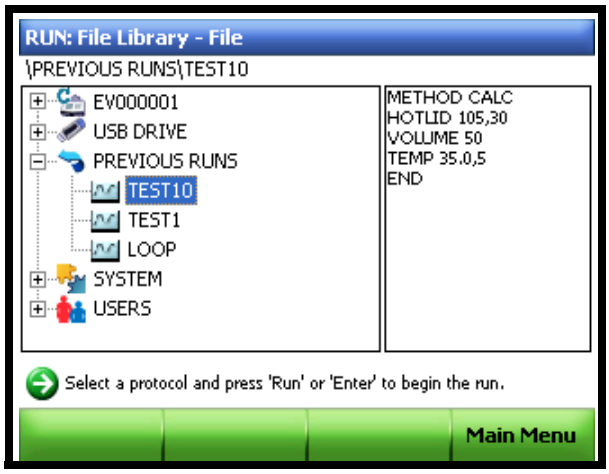
Start a run using one of the following methods:

- **Press the** RUN **key: Press** RUN**, and select a protocol in the file library. Press** RUN **or** ENTER **again to begin the run sequence.**
- **Select a protocol in the** PREVIOUS RUN **folder and press** RUN**: Select the** Previous Run **folder to select a protocol that was recently run. Then press the** RUN **or** ENTER **key to rerun the selected protocol.**
- **Open the file library to select a protocol: Click** Files **in the main menu to open the file library and select a protocol. Press the** RUN **or** ENTER **key to run the selected protocol.**
- **Run an edited protocol: After editing a protocol, press the** RUN **key to begin running that protocol.**
- **Run a new protocol: After creating a new protocol (page 47), press the** RUN **key to begin running that protocol.**

To run a protocol by pressing the RUN **key, follow these steps:**

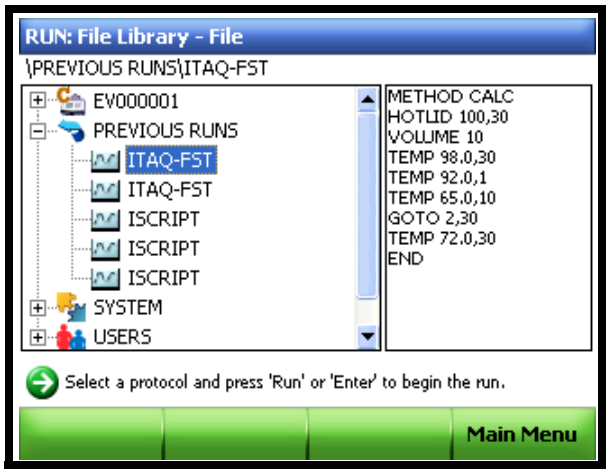
1. **Press** RUN **and select a folder that contains a protocol**

   Press the RUN **key from any screen to open the file library and select a folder:**



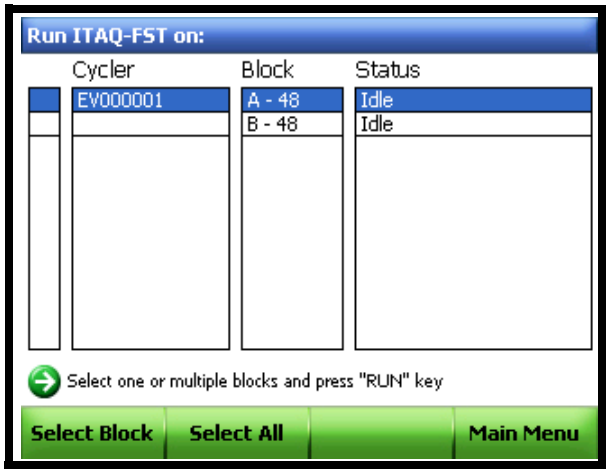
2. **Select a protocol and press** RUN **to initiate the run**

   Select a protocol and press the RUN **key again to begin running the selected protocol. In this example, the** ITAQ-FST **protocol is selected:**



3. **Choose one or more blocks to run (optional)**

   When more than one block is connected to the C1000, choose the blocks you want to run the protocol. In this example, the C1000 is running a Dual-48 reaction module:



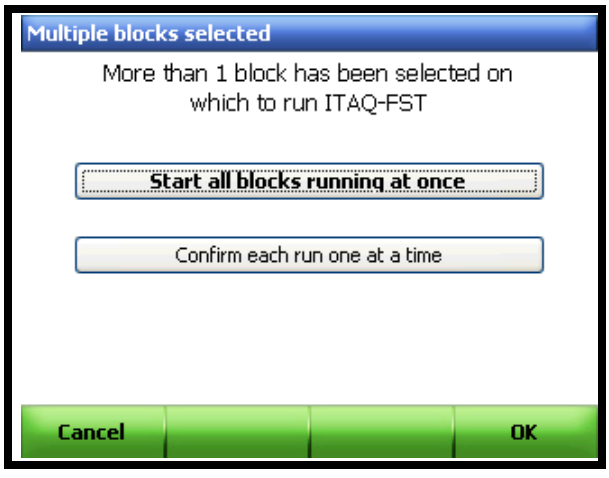
   TIP: To select more than one block, first select one block by pressing the arrow keys and pressing `Select Block`. Then, select the next block by pressing the arrow key and pressing `Select Block` again.

4. **Choose a method to start multiple blocks (optional)**

   When you choose to run a protocol on more than one block, first choose a method, and then press `OK` to begin the run.

   - **Start all blocks running at once: Choose this method to begin running the protocol on all blocks at the same time.**
   - **Confirm each run one at a time: Choose this method to begin running the protocol on one block at a time.**

   In this example, the protocol will start running on all of the blocks at the same time:

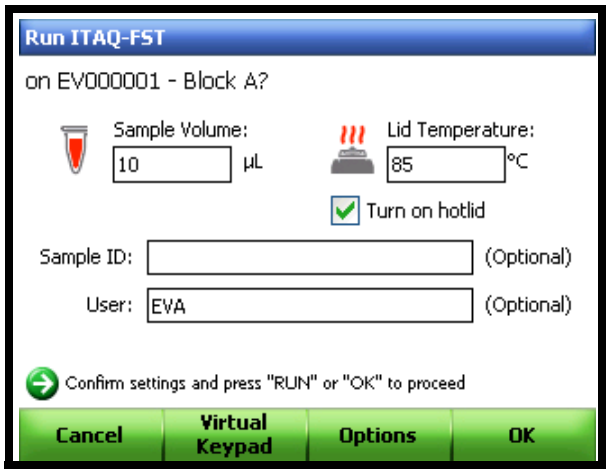


5. **Confirm the run parameters**

   Before starting the run, confirm or change the run parameters. The sample volume and lid temperature parameters influence the success of your reaction. For more information about selecting these parameters see page 64.

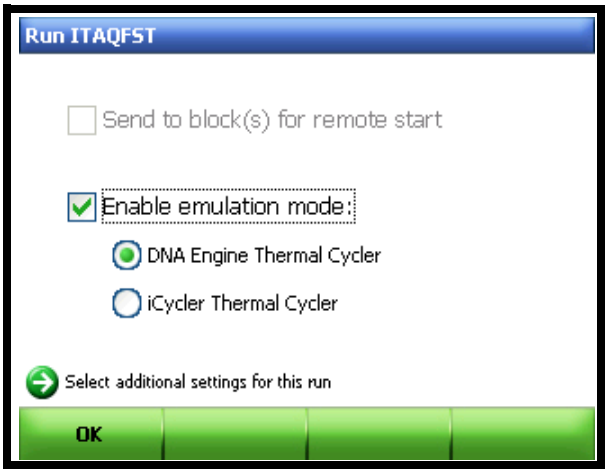
   TIP: The sample volume entry determines the temperature control mode. Enter a `Sample Volume` **greater than 0 (zero) uL for Calculated temperature control, in which the thermal cycler uses the sample volume to calculate the target temperature. Enter 0 (zero) to use Block temperature control, in which the thermal cycler assumes the sample is the same temperature as the block temperature.**

   In this example, the run parameters include the user EVA, **the sample volume, and lid temperature 85°C: (It is also possible to bypass these fields and run the protocol without entering parameters or Sample ID by pressing Run).**



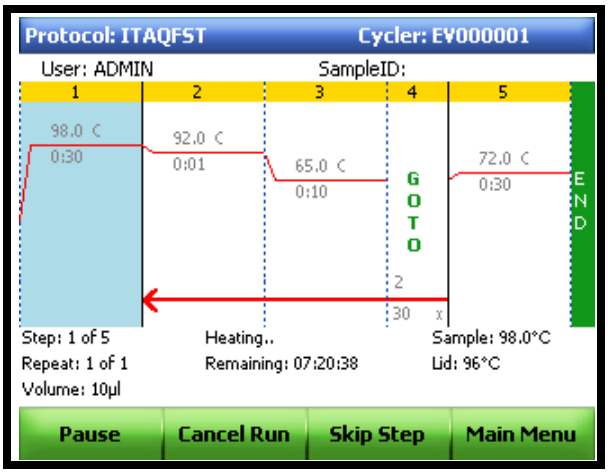
6. **Run in emulation mode (optional)**

   To run a protocol at the same ramp speed as the iCycler and DNA engine select the emulation option. Press the `Options` **button, then select the** `Enable` **emulation mode and specify the ramp rate desired:**



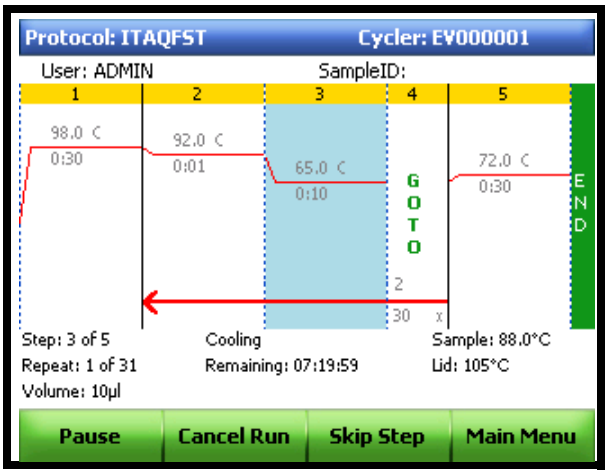
7. **Start the run**

   Press OK to start the run. In this example, the protocol run is starting on block A, and the lid is preheating:



8. **Monitor the protocol as it runs**

   Once the protocol begins to run, the Status screen provides details about the progress of the protocol. In this example, the thermal cycler is cooling the sample to the target temperature of 65.0°C:



Monitoring a Run With the STATUS and VIEW Keys

During a run, the STATUS and VIEW keys provide quick ways to monitor all blocks:

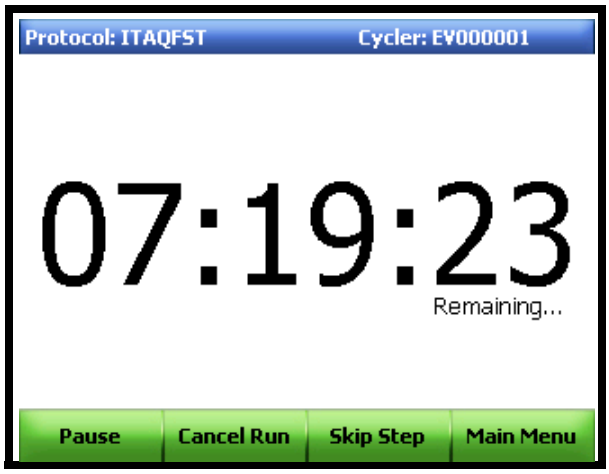
- **VIEW key: Press this key to see the time remaining on one block.**
- **STATUS key: Press this key to see the status of runs on multiple blocks.**

Monitoring With the VIEW Key

When running one block, press the VIEW key to switch between the time remaining screen and the detailed protocol run screen. To monitor the protocol using the VIEW key, follow these steps:

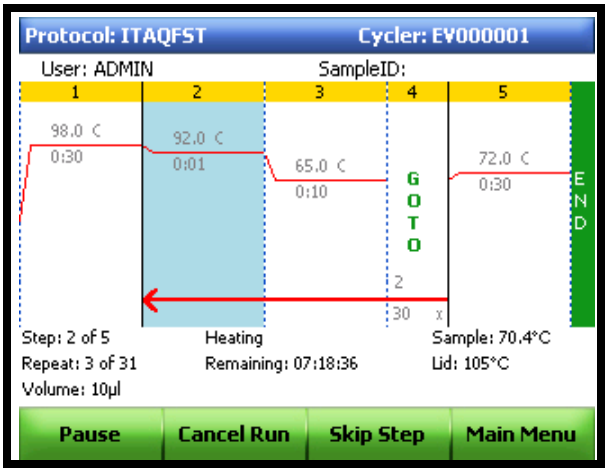
1. **Press VIEW to monitor the remaining time.**

   Press the VIEW key to switch from the run screen to the time remaining screen:



2. **Press VIEW again to return to the run screen**

   Press the VIEW key again to switch back to the run screen:



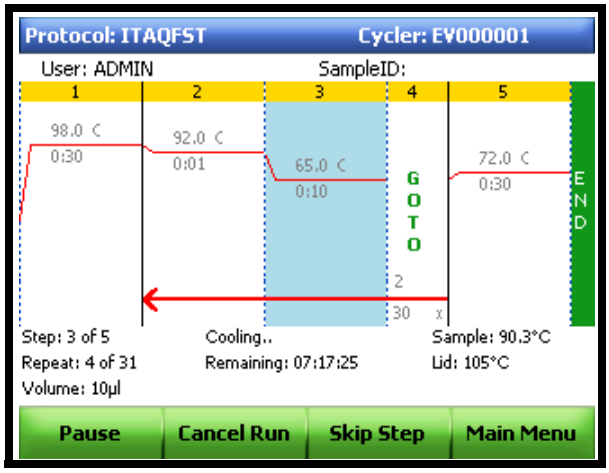
TIP: The main menu also lists the status of all blocks connected to the C1000.

Monitoring Multiple Blocks With the STATUS Key

When you monitor runs on multiple blocks, press the STATUS key to view the status of all blocks connected to the C1000 and scroll through these screens:

1. **Monitor the run on one block**

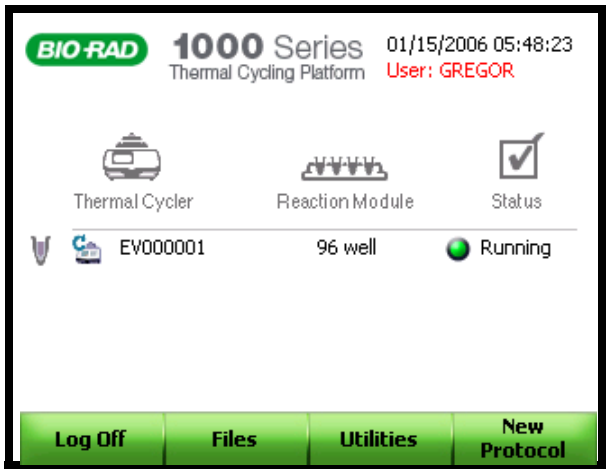
   Monitor the details of a run on one block in the protocol screen. This screen provides the details about the current run:



   Select one of these buttons to change the current run:
   - **Press `Pause` to temporarily stop the current run.**
   - **Press `Skip Step` to skip the step that is currently running.**
   - **Press `Cancel` to cancel the current run.**

2. **Press the `Main Menu` key to monitor all blocks on the main menu screen**

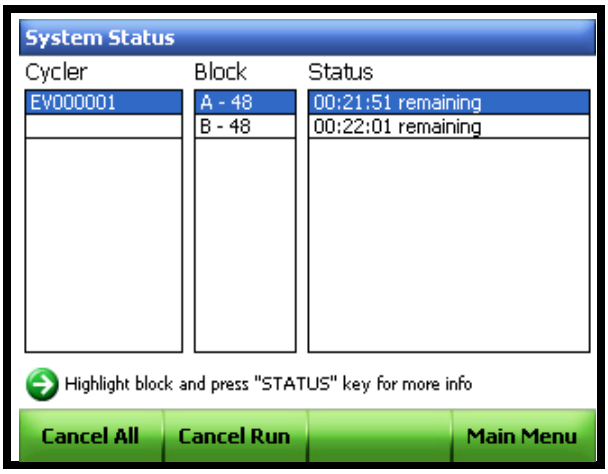
   View the status of one or more blocks in the main menu. This screen displays a list of all the connected blocks and their current status. In this example, the C1000 thermal cycler is running one 96-well block:



3. **Press the `STATUS` key to monitor multiple blocks on the block list screen (optional)**

   The block list screen is displayed when the C1000 is running multiple blocks. This screen provides an overview of the status of all the blocks connected to the

C1000. In this example, the C1000 is running a Dual-48 reaction module and the block status screen displays the two blocks:



TIP: Press the STATUS key again to enter the protocol screen for the selected block.

Pausing and Resuming a Run With the Pause Button

Select the Pause button to temporarily pause a running protocol. During a pause, the thermal cycler continues heating or cooling to target temperature, and the lid temperature is maintained.

TIP: After a power failure, the C1000 thermal cycler will automatically resume running a protocol, but will also display a warning message.

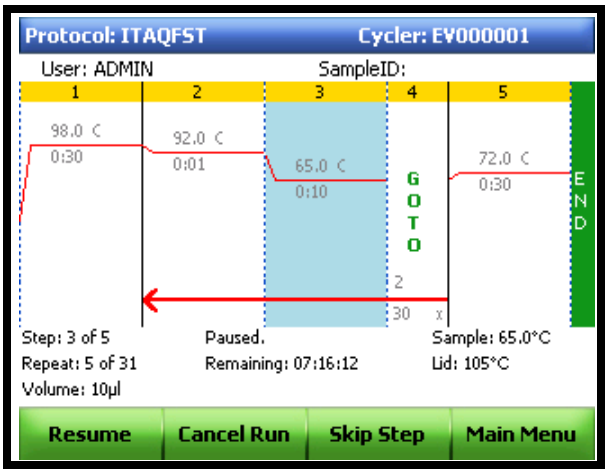
To pause and resume a running protocol, follow these steps:

1. **Pause the protocol at the current step**

   Press the Pause button, from the graphical display of the running protocol, to stop the protocol.

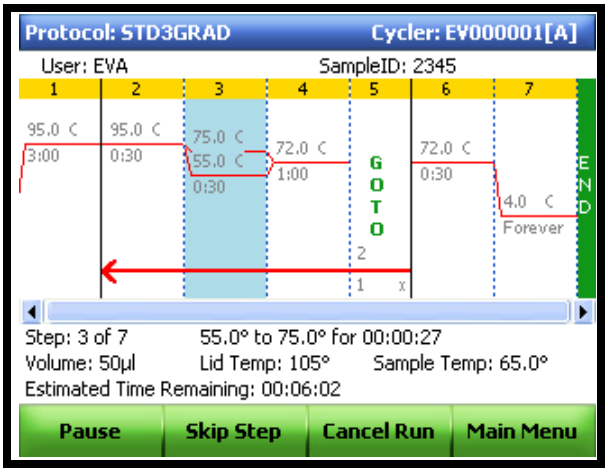
   WARNING! Pausing a step can adversely change the results of your PCR reaction. If you pause the protocol during a temperature step, the PCR reaction stays at the target temperature for a longer hold time than the protocol step requires.

In this example, the protocol is paused at step 3:



2. **Resume the protocol**

   Press the `Resume` button to start running the paused protocol step. In this example, step 3 is continuing to run.



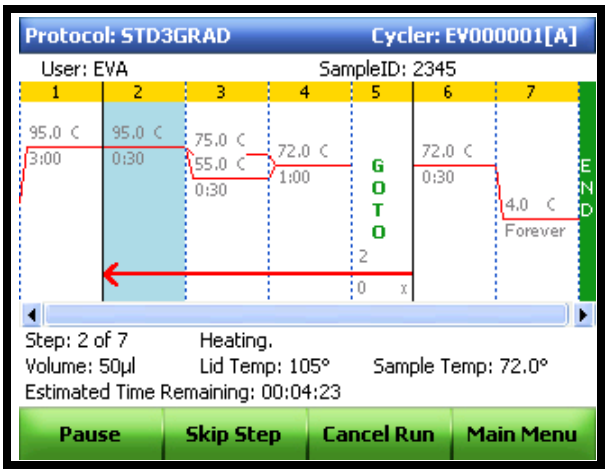
Skipping Protocol Steps With the `Skip Step` Button

Skip a step if it is necessary to shorten a protocol while it is still running. By repeatedly skipping steps, it is possible to skip several GOTO loops and shorten a running protocol.

To skip the current protocol step, follow these steps:

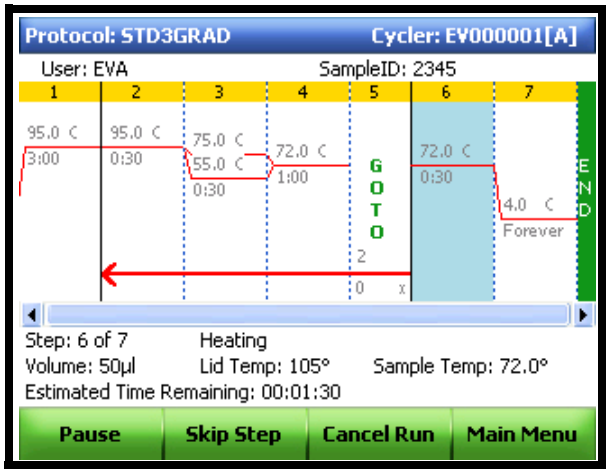
1. **View the current step**

   In this example, step 2 is currently running, and the last GOTO repeat in the protocol is running (`0x`):



2. **Select `Skip Step`**

   Press the `Skip Step` button to skip the current step. Press `Skip Step` multiple times to skip more than one step. In this example, `Skip Step` was pressed twice, skipping step 3 and 4 in the last GOTO loop. Therefore, the C1000 finished the GOTO repeats, and is running the next step (6):



Stopping a Run With the `Cancel Run` Button

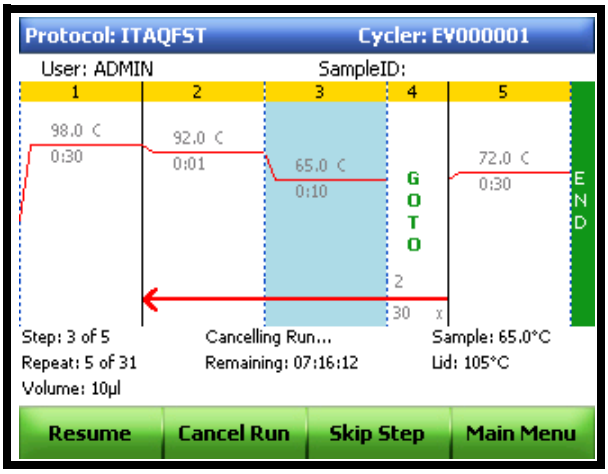
If it is necessary to cancel a run protocol at any time, click the `Cancel Run` button in the protocol screen. When the protocol is cancelled, the block immediately stops changing temperature. To cancel a protocol, choose one of these two screens:

- **Protocol screen: Choose this screen to cancel a protocol on a single block.**
- **Block status list: Choose this screen to cancel a protocol on one or more blocks.**

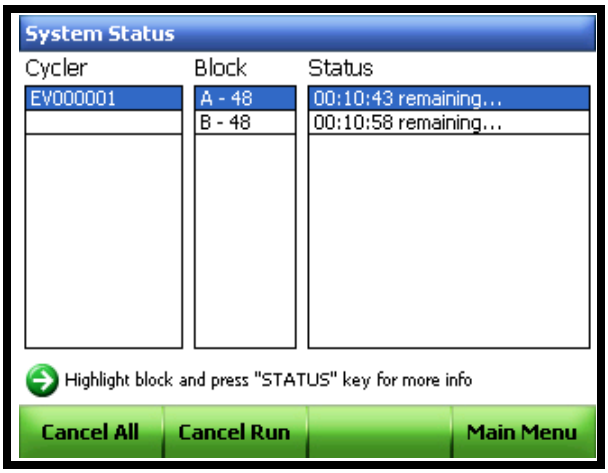
  NOTE: Do not turn the thermal cycler off immediately after cancelling a run. The fans might need to run in order to cool the block.

1. **Open the protocol screen or the block status list**

   To cancel a protocol on a single block, open the protocol run screen and select `Cancel Run`**. In this example, one block is running and the protocol screen is open:**

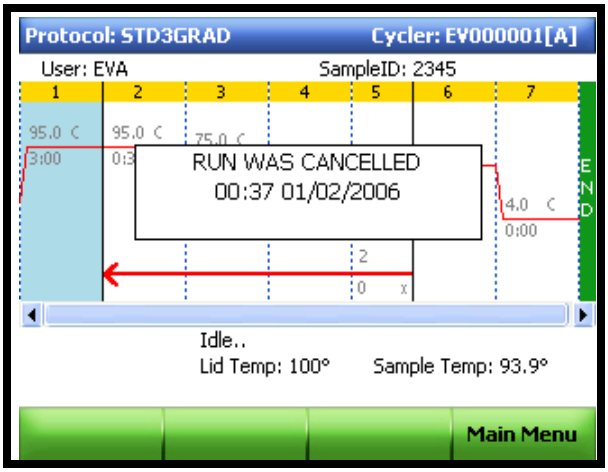


   In this example, multiple blocks are running and the block status screen is open:

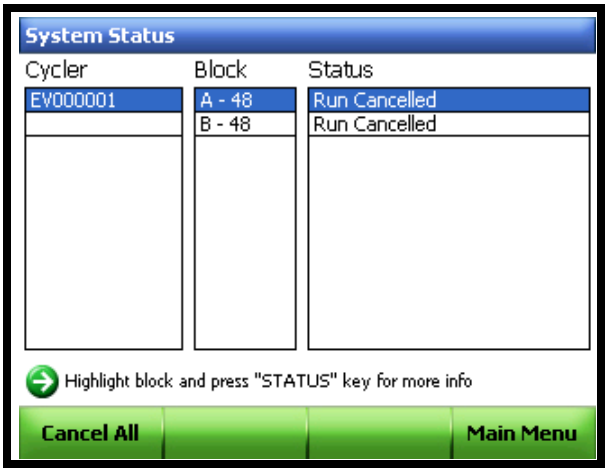


2. **Click** `Cancel Run` **or** `Cancel All`

   Click the `Cancel Run` **button to stop the run on a single block. In this example the protocol screen is open and the run is cancelled:**



   In this example, the block status screen is open and the run is cancelled:



   TIP: To cancel multiple runs, but not all, select the blocks and click `Cancel Run`**.**

Incubating Samples With the `INCUBATE` Key

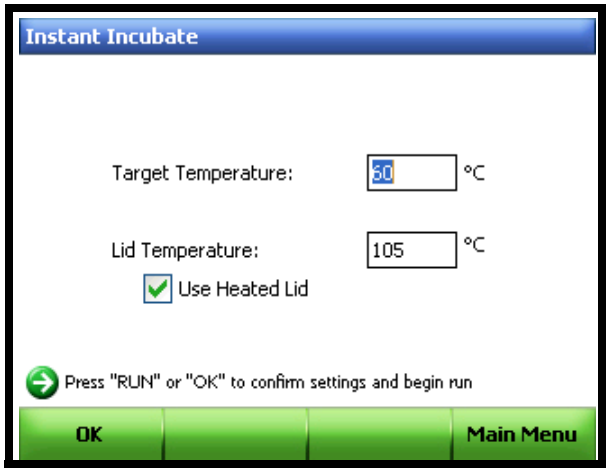
Click the `INCUBATE` **key (0) to keep samples at a constant temperature for any amount of time. The incubation will continue indefinitely. To end an incubation, press the** `Cancel` **button.**

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

Follow these steps to start and end incubating samples at a single temperature:

1. **Press** `INCUBATE`

   Load your samples and press the `INCUBATE` **key (0) to initiate an incubation:**

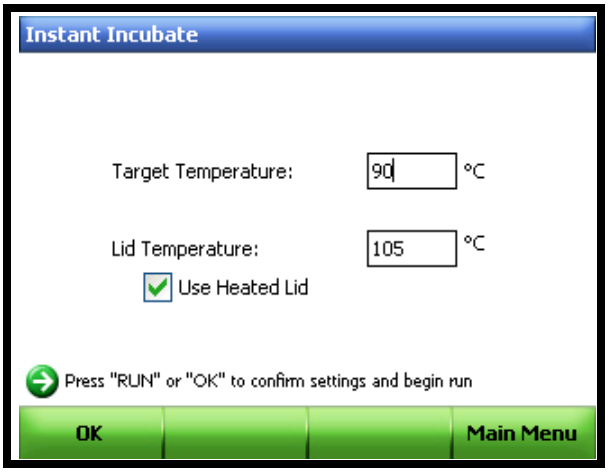


   TIP: Set the default incubation temperature in the `Utilities` **menu (page 101).**

   NOTE: When incubation is occurring at temperatures below `Cutoff`**, the lid will maintain a temperature of 31°C to prevent excessive condensation. The** `Cutoff` **may be changed from the** `Utilities` **menu.**

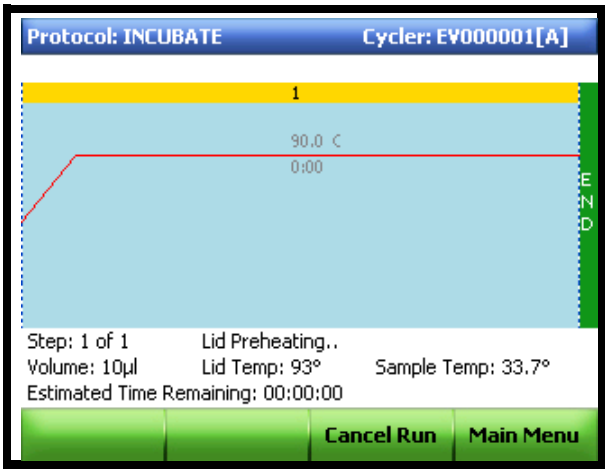
2. **Enter the parameters for the incubation**

   Change the `Target Temperature` **and** `Lid Temperature` **parameters as needed. In this example, the Target Temperature is changed to 90°C:**



3. **Press `OK` to start the incubation**

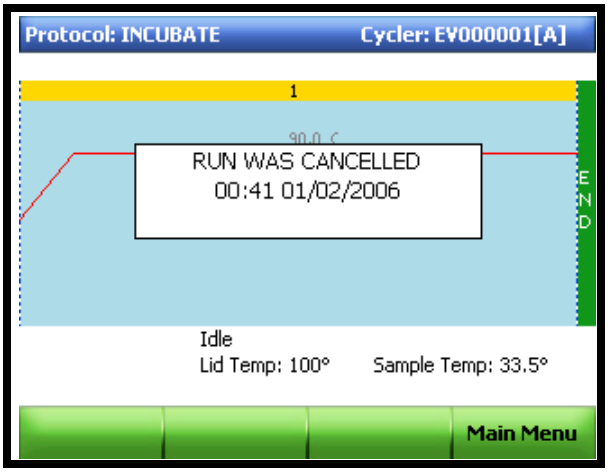
   Press the `OK` button twice to start the incubation:



   NOTE: If multiple blocks are connected, you may select which blocks you want to run the incubation.

4. **Press `Cancel Run` to end the incubation**

   Press the `Cancel Run` button to end the incubation and view the final "Cancelled" screen:



6 Create and Edit Protocols

Both creating and editing protocols are easy and intuitive. When creating a new protocol without the help of Autowriter, the C1000™ thermal cycler provides a template protocol.

> **TIP: To have the C1000 thermal cycler automatically create a protocol, press the Protocol Autowriter key ("Creating a Protocol With the Protocol Autowriter" on page 24).**

This chapter includes instructions on how to create and edit protocols. The process of creating and editing protocols includes both required and optional tasks; after understanding these simple steps, protocols can be created utilizing these tasks in any order:

1. **Open a new protocol or an existing protocol (below).**
2. **Change the parameters in a temperature or gradient step (optional, page 48).**
3. **Change the parameters in a `GOTO` step (optional, page 57).**
4. **Insert or delete a step (optional, page 59).**
5. **Name the protocol (required, page 62).**
6. **Change the sample volume and the lid temperature (optional, page 64).**
7. **Save the protocol (required, page 66).**

For more information about using the control panel and main menu (page 13). For more information about the content of protocol files, see page 79. For instructions about running a protocol, see page 33.

Opening a New Protocol or an Existing Protocol

Follow these instructions to begin creating or editing a protocol:

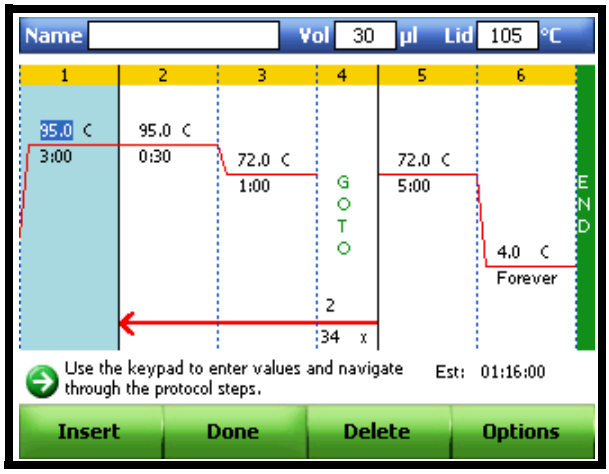
1. **Select `New Protocol`, or press the `EDIT` key.**

   To create a new protocol, select `New Protocol` from the main menu to open a new protocol template. To change an existing protocol, press the `EDIT` key to open the file library and select a protocol to edit.

2. **Switch between graphic and text view (optional).**

   A protocol opens in graphic view. Press the `VIEW` key to switch between graphic view and text view at any time:

   - **This is the new protocol template in graphic view:**



   - **This is the template protocol in text view:**

Changing Parameters in a Temperature Step

Temperature steps are the most common variety in a typical protocol. Temperature step parameters include the target temperature and the hold time, and these are required to complete a step. In addition, you can add more parameters to a temperature step by clicking the `Options` button.

The following list includes all the possible parameters for temperature steps. Many of these parameters can be combined in a single step:

- **Temperature and Time (required): Change target temperature and the hold time in a step (below).**

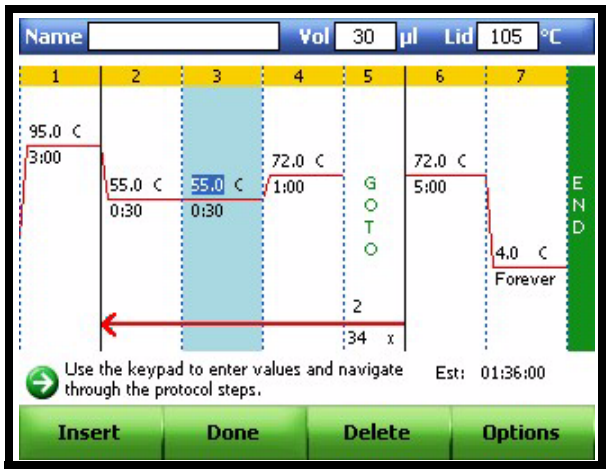
- **Gradient: Create a temperature gradient across the block during the step (“Adding and Removing a Temperature Gradient” on page 50).**
- **Increment: Change the target temperature with each cycle (page 51).**
- **Ramp Rate: Change the ramp rate for the step (page 53).**
- **Extend: Extend the hold time in the step with each cycle (page 54).**
- **Beep: Add a beep at the end of the step (page 56).**

Changing Target Temperature and Hold Time

To change the target temperature and the hold time in a temperature step, follow these instructions:

1. **Select a protocol step**

   This example shows the default template for a new protocol. Notice that each temperature step includes a target temperature and a hold time:

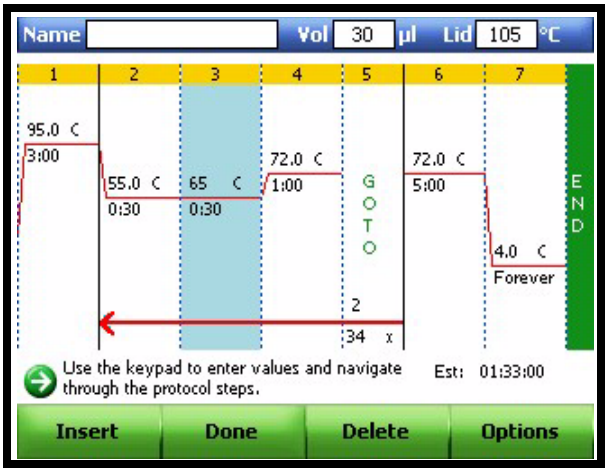


2. **Enter desired parameters**

   Press the arrow keys to select a parameter. Press the alphanumeric keys to enter a new number for any parameter highlighted.

   TIP: To change between graphic view and text view, press the `VIEW` **key.**

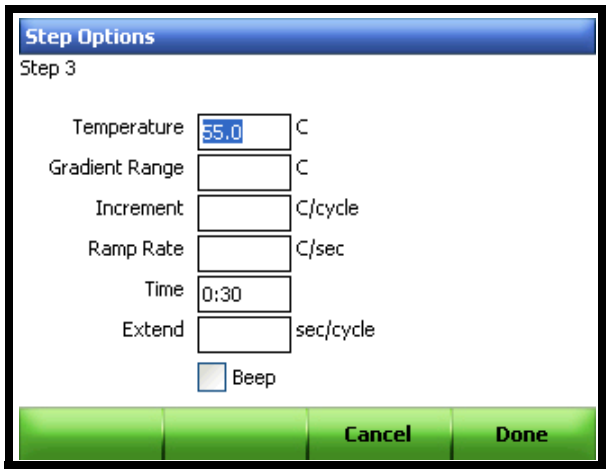
In this example, the target temperature in step 3 is changed to 65°C:



Adding and Removing a Temperature Gradient

To add a temperature gradient to a temperature step, follow these instructions:

1. **Select a protocol step and select** `Options`, **or press** `Insert`, **then** `Gradient`
   Select the temperature step, and press `Options` **to open the Options window:**



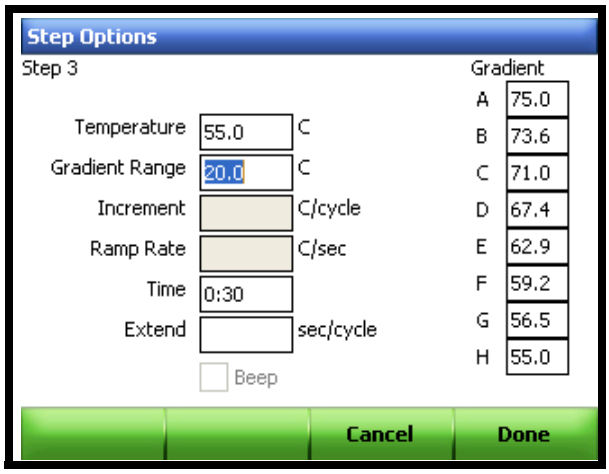
2. **Enter the Gradient Range**

   Press the alphanumeric keys to enter a `Gradient Range`**. Press** `Done` **to continue.**

   NOTE: A gradient range must be between 1 and 24°C. The highest temperature in the gradient is the lower temperature plus the gradient range.

   In this example, the gradient range is 20.0°C and the low temperature is 55°C. Thus, the upper temperature is 75°C. Once the gradient range is entered, the screen displays the gradient distributed across the rows of wells in the block. In

this example, the gradient is displayed across a 96-well block from the back (row A, upper temperature) to the front (row H, lower temperature):



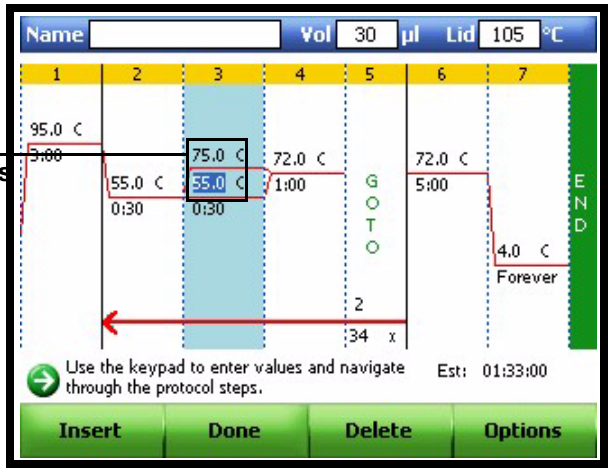
NOTE: A gradient step can also include an Extend parameter, but not an Increment, Beep or Ramp Rate.

3. **View the gradient**

   Review the protocol in the preferred view (text or graphical). This is also another chance to edit the gradient parameters.

   TIP: Once a step has a gradient, you can edit the upper and lower temperatures in the graphic or text view without opening the Options screen

   In this example, the gradient is from 55 to 75°C:



4. **Remove the temperature gradient**

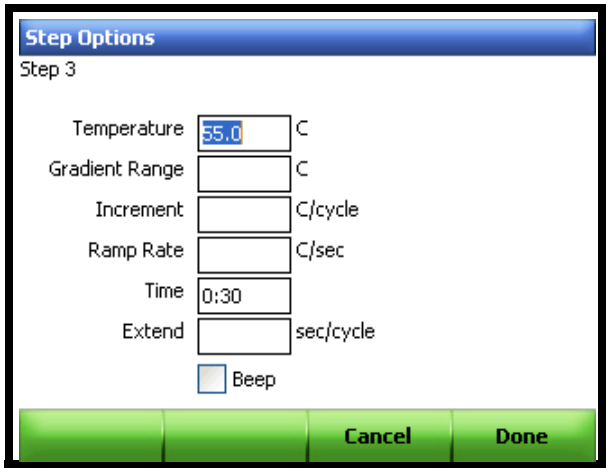
   Select the gradient step, and open the `Options` **window. Then select the** `Gradient Range` **and delete the range by pressing the** `BACK` **key. Then press** `OK`**.**

Adding or Removing a Temperature Increment

An increment raises or lowers the target temperature for a step with each cycle. To add and remove a temperature increment in a temperature step, follow these instructions:

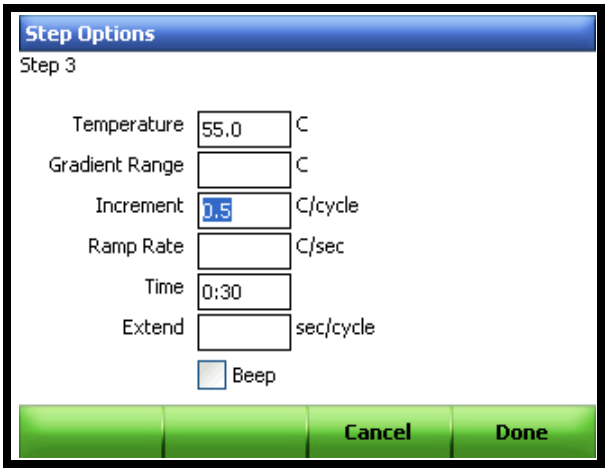
1. **Select a protocol step and click** `Options`

   Select the temperature step, and press `Options` **to open the Options window:**



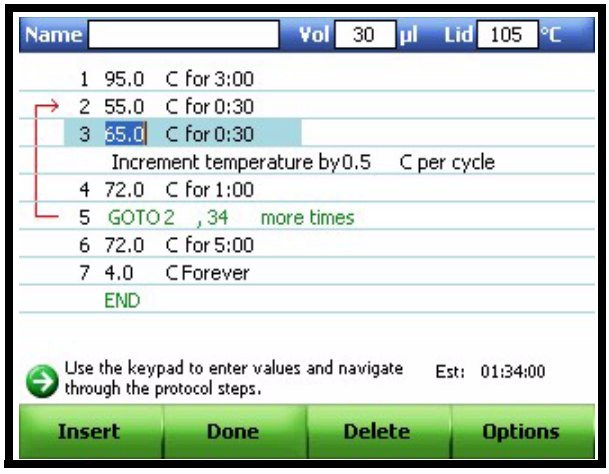
2. **Add an increment**

   Press the arrow keys to select the `Increment` **box. Then press the alphanumeric keys to enter an increment temperature, and press** `OK` **to finish:**



3. **View the increment**

   View the increment in the text view of a protocol. In this example, protocol step 3 has an increment:



4. **Remove an increment**

   Select the protocol step and open the Options window. Then delete the `Increment` **temperature by pressing the** `BACK` **key. Then press** `OK`**.**

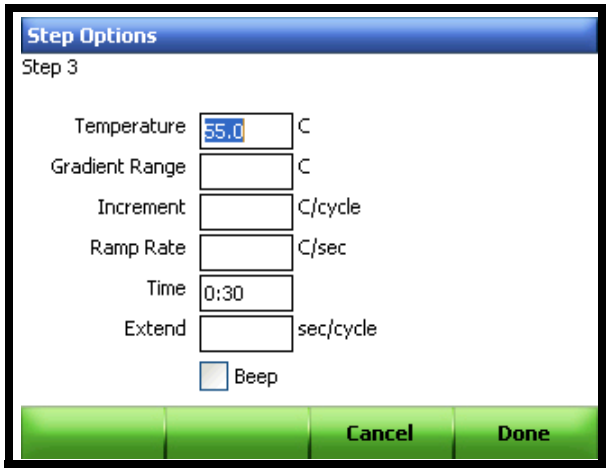
Adding and Removing Ramp Rate

Change the ramp rate of a protocol step when you need to emulate a thermal cycler that runs at a slower ramp rate than the C1000.

To change the ramp rate for a temperature step, follow these instructions:

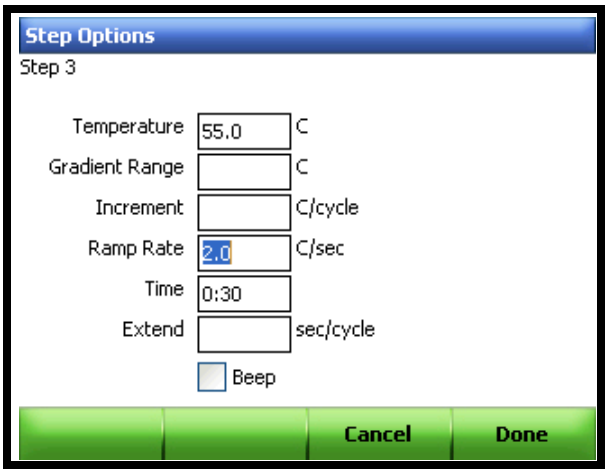
1. **Select a protocol step and select** `Options`

   Select the temperature step, and press `Options` **to open the Options window:**



2. **Add a ramp rate change**

   Press the arrow keys to put the cursor in the `Ramp Rate` box. Finally, press the alphanumeric keys to enter a ramp rate and press `OK` to add the specified ramp rate:



3. **View the ramp rate**

   The ramp rate is seen in the text view of a protocol. In this example, protocol step 3 has a ramp rate change:

4. **Remove a ramp rate change**

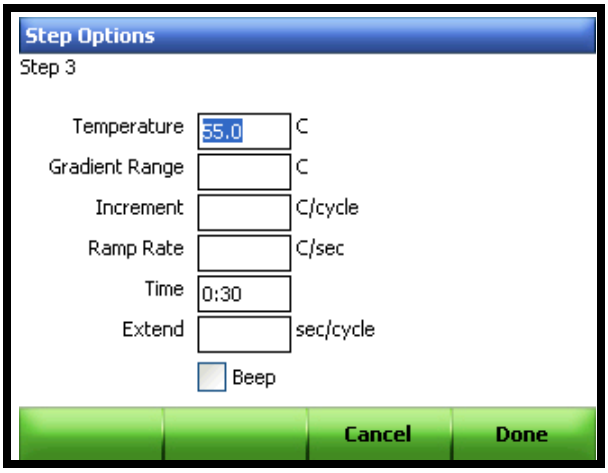
   To remove a ramp rate change, select the step and open the Options window. Then select ramp rate and delete the ramp rate by pressing the `BACK` key. Then press `OK`.

Adding and Removing an `Extend` Time

Add an extend time to a protocol step when you want the hold time to increase or decrease with each cycle. To add and remove an extend time, follow these instructions:

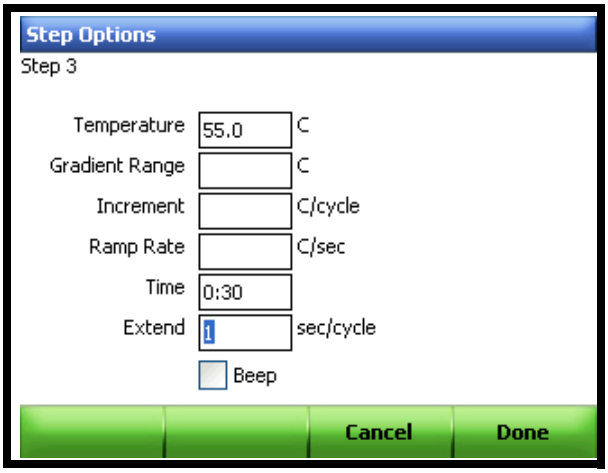
1. **Select a protocol step and click** `Options`

   Select the temperature step, and press `Options` **to open the Options window**:



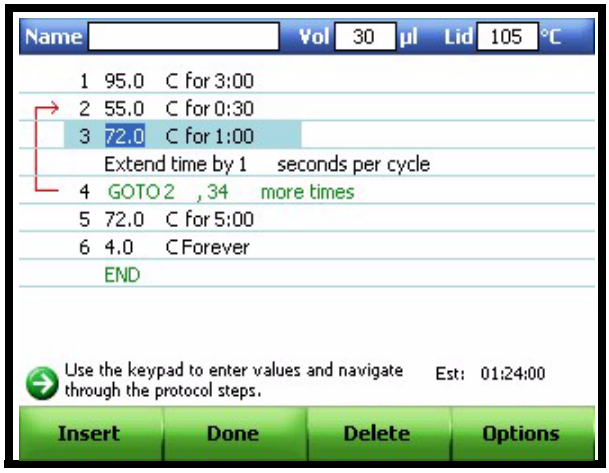
2. **Add an extend time**

   Press the arrow keys to put the cursor in the `Extend` **box. Finally, press the alphanumeric keys to enter an extension time and press** `OK` **to add the extension:**



3. **View the extend time**

   An extend time is seen in the text view of a protocol. In this example, protocol step 3 has an extend time:



4. **Remove an extend time**

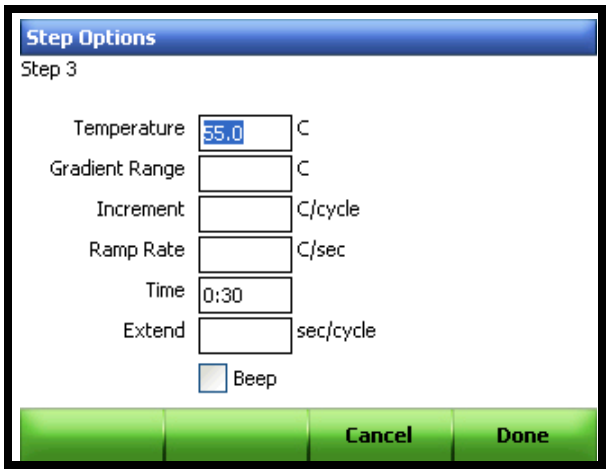
   To remove an extend time, select the step and open the Options window. Then delete the extend time by pressing the BACK **key. Then press** OK**.**

Adding and Removing a Beep

A beep is a sound that the thermal cycler emits when it reaches a protocol step. To add or remove a beep follow these instructions:

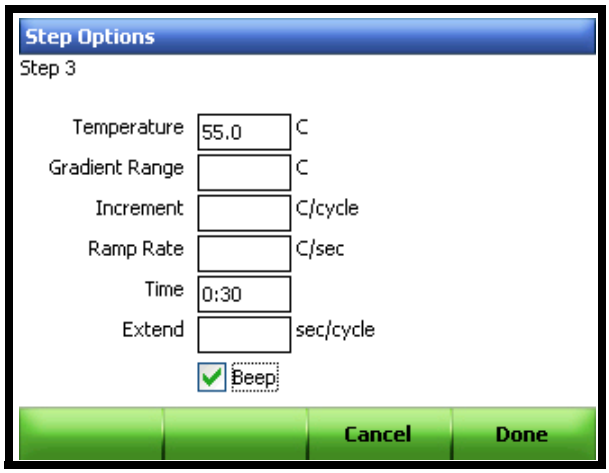
1. **Select a protocol step and click** Options

   Select a temperature step, and press Options **to open the Options window**:



2. **Add a Beep**

   Press the arrow keys to highlight the word "Beep". Finally, press ENTER **to select the box and press** OK **to confirm:**



3. **View the Beep**

   View the beep in the text view of a protocol. In this example, protocol step 3 has a beep added:

4. **Remove a Beep**

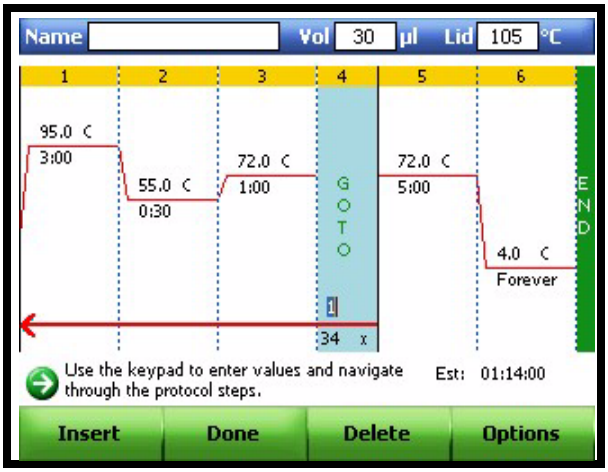
   To remove a beep, select the step and open the Options window. Then delete by pressing the ENTER **key after selecting** Beep**. Then press** OK**.**

Changing Parameters in a GOTO Step

The GOTO step instructs the thermal cycler to repeat a set of steps in a loop. This step creates a cycle in the PCR experiment. To change parameters in a GOTO step, follow these instructions:

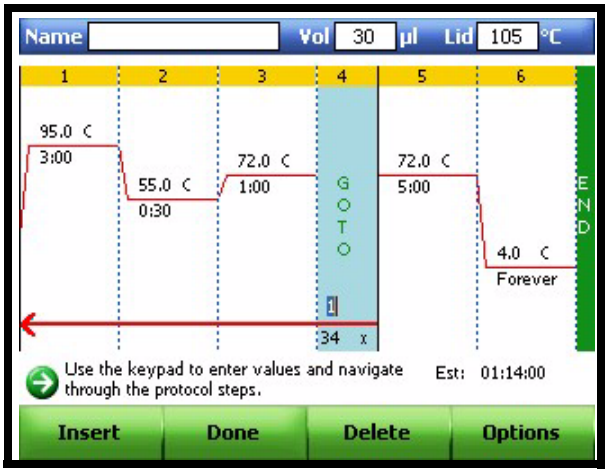
1. **Select a GOTO step.**

   Select a GOTO step in the graphic or text view. In this example, the step parameters include a GOTO step 1, and 34 additional repeats. Notice that the GOTO arrow points to step 1:



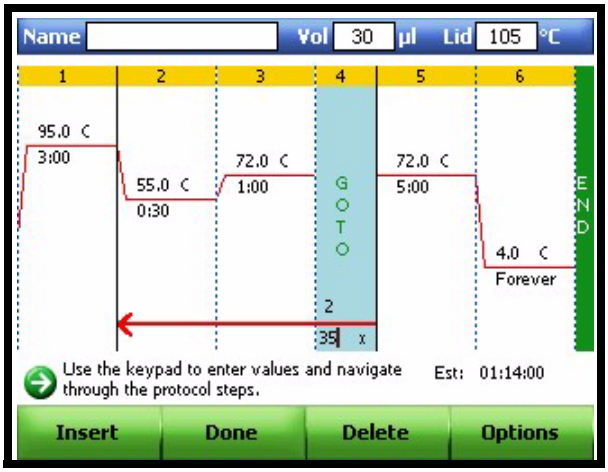
2. **Change the GOTO step number**

   Press the arrow keys to select the step number in a GOTO step. In this example, the GOTO step is changed from step 1 to step 2. Notice that the GOTO arrow moved to step 2:



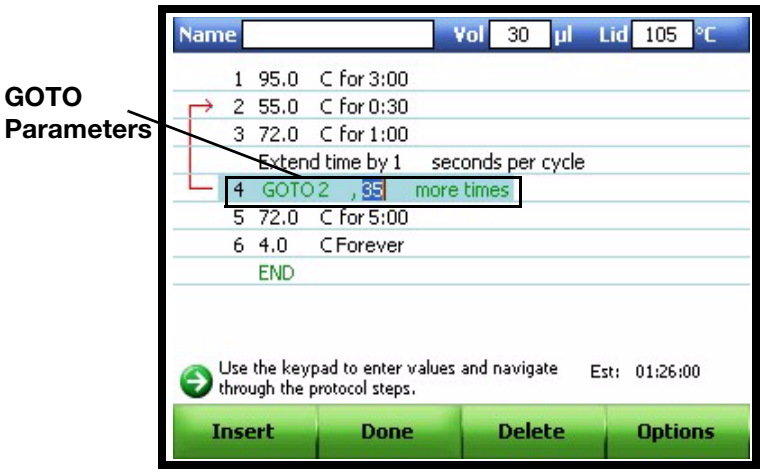
3. **Change the GOTO loop**

   The GOTO loop is the number of repeated sets of steps in the GOTO step. In this example the number of GOTO loops is changed from 34x to 35x:



4. **View the GOTO parameters in text view**

   View the changes in text view or graphic view. In this example, the protocol is in text view:



Inserting and Deleting a Step

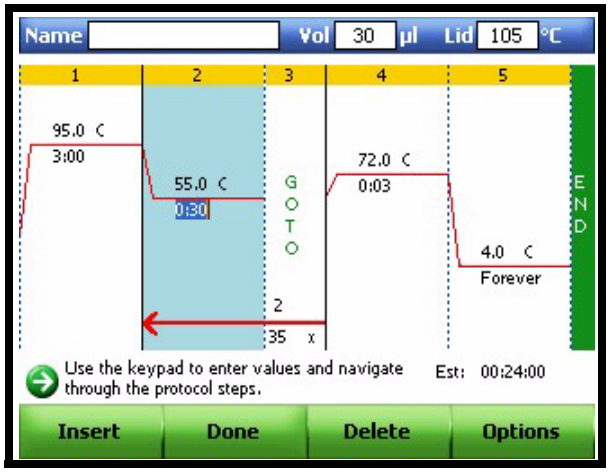
Insert a protocol step if a new temperature (`Temp`), GOTO, or Gradient step is needed. Delete a step to remove it from the protocol.

Inserting a Protocol Step

Follow these instructions to insert a step to the right of a preexisting protocol step:

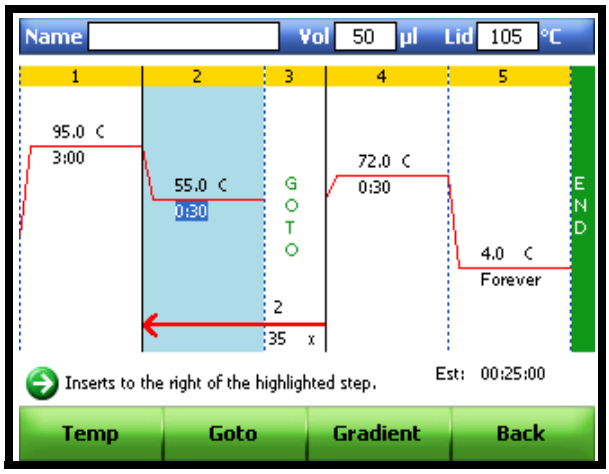
1. **Select a position to insert the new step and select** `Insert`

   Select a step to the left of where you want to insert a step. Then click `Insert` **to begin inserting a step. In this example, step 2 is selected:**



2. **Choose the type of step to insert**

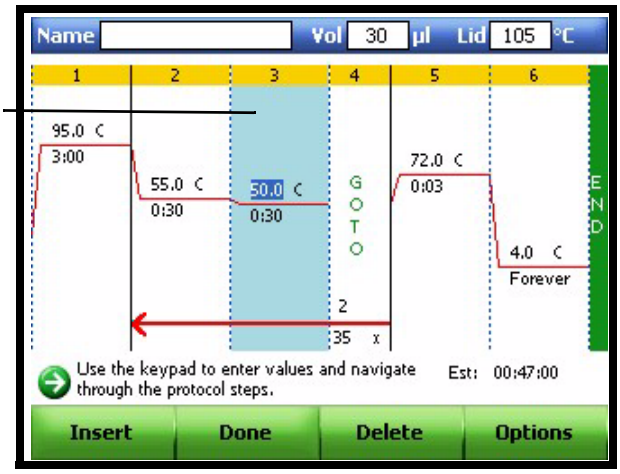
   Select `Temp` **to insert a temperature step;** `GOTO` **to insert a GOTO step; or** `Gradient` **to insert a gradient step:**



3. **Edit the new step parameters**

   The new step has a default target temperature of `50`**°C, and a default time of** `30` **seconds (0:30). Select and edit the parameters in the new step (Step 2 on**

page 49). In this example, a temperature step is inserted. Notice that the step is inserted inside a GOTO repeat:

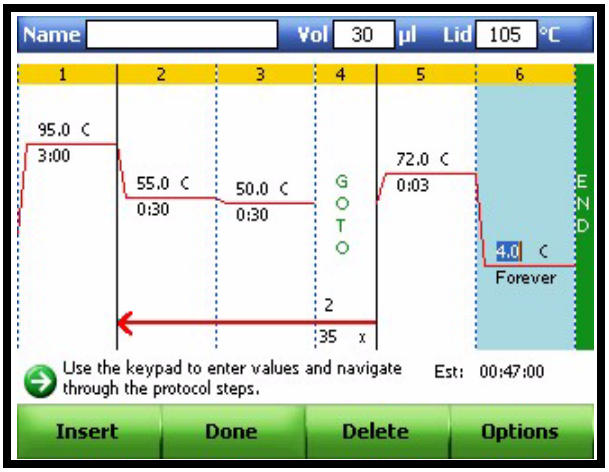


Delete a Protocol Step

To permanently remove a step from a protocol, delete the step by following these instructions:

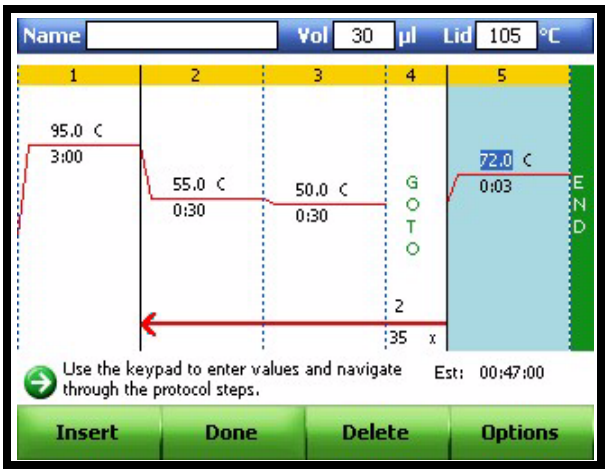
1. **Select a step to delete**

   Select a step. In this example, step 6 is selected:



2. **Press** `Delete`.

   Press `Delete` **to remove the selected step. In this example, step 6 was deleted and step 5 is now the final step in the protocol:**



Naming a Protocol

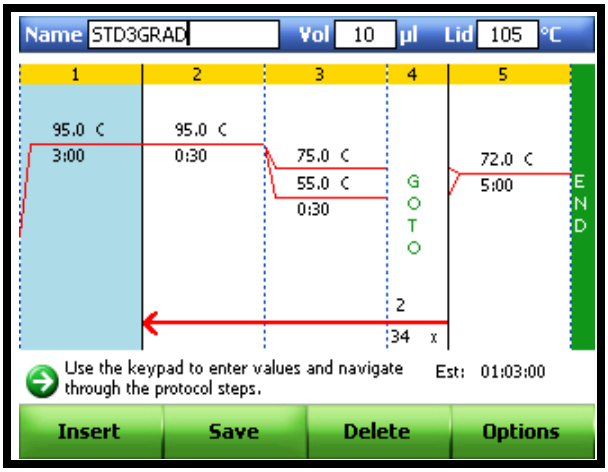
A new protocol requires a name, which can later be changed, if so desired, when editing the protocol.

Naming a New Protocol

To name a protocol, follow these steps:

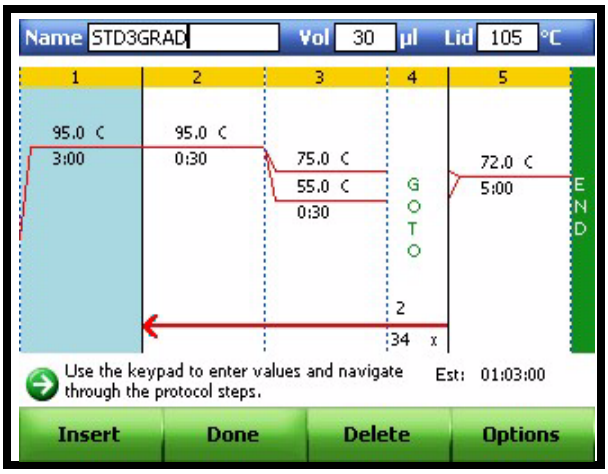
1. **Select the protocol** `Name` **box.**

   Press the arrow keys to move the cursor to the Name box:



2. **Enter a name**

   Type the name of the protocol in the box. Press the alphanumeric keys to enter a letter or number. Press `Done` to accept the name and continue to the file library. In this example, the new protocol name is STD3GRAD:



NOTE: A protocol name can include up to eight letters and numbers. It cannot be duplicated in the same folder or contain spaces, dots, or dashes.

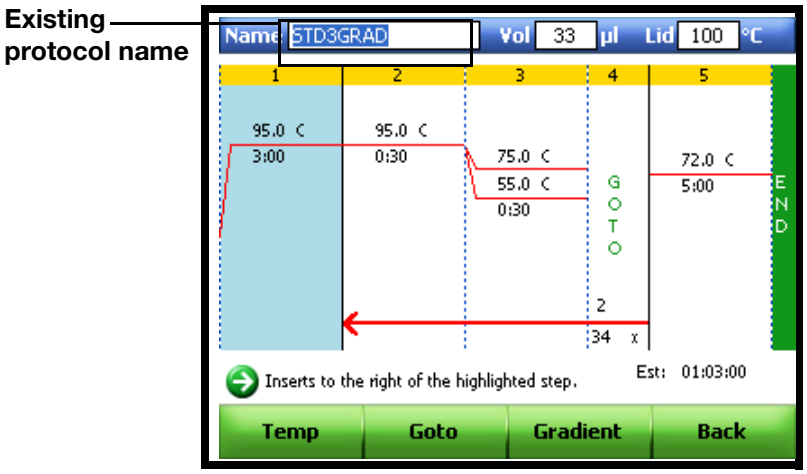
Renaming an Existing Protocol

When you edit an existing protocol, you have the option of renaming the protocol. Renaming the protocol does not remove the original protocol and name.

To rename a protocol, follow these steps:

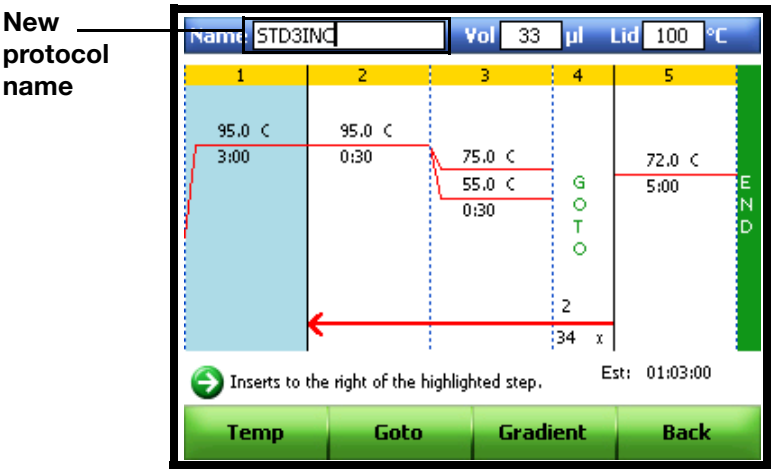
1. **Select the name in the `Name` box**

   Press the arrow keys to select the protocol name in the `Name` box. In this example the name of the existing protocol is STD3GRAD:



2. **Type the new protocol name**

   Press the BACK **key to delete the name. Then type the new name in the** Name **box. Press the alphanumeric keys to enter a letter or number. Press** ENTER **to accept the name. In this example, the new name is STD3GRAD:**



Change the Sample Volume and Lid Temperature

The parameters for sample volume and lid temperature influence the success of a protocol. The C1000 thermal cycler offers three ways to set the sample volume and lid temperature in a protocol:

- **Set the default protocol parameters when creating and editing the protocol (below).**
- **Change the** DEFAULT **protocol within the** SYSTEM/TEMPLATE **folder.**
- **Set these parameters while initiating a run (page 33)**

Changing the Sample Volume

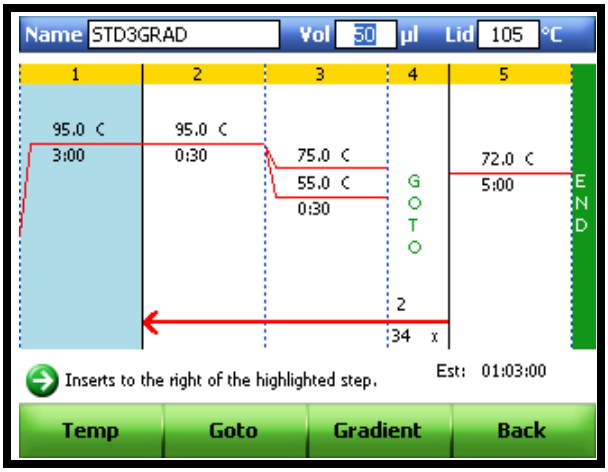
Enter the volume of your samples, or enter zero. The sample volume you enter determines the temperature control mode during a run (“Temperature Control Mode” on page 70).

To change the sample volume, follow these steps:

1. **Select the sample volume**

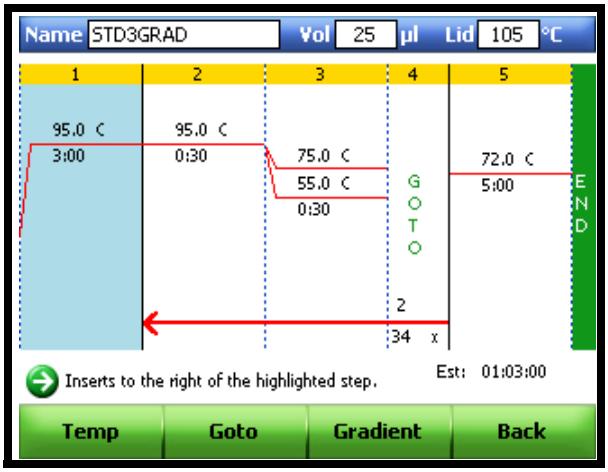
   To change the default sample volume for this protocol, select the sample volume box (Vol**).**

In this example, the default sample volume for a new protocol is `50` microliters:



2. **Enter a sample volume**

   Press the alphanumeric keys to enter a new sample volume. In this example, the new sample volume is `25` microliters:



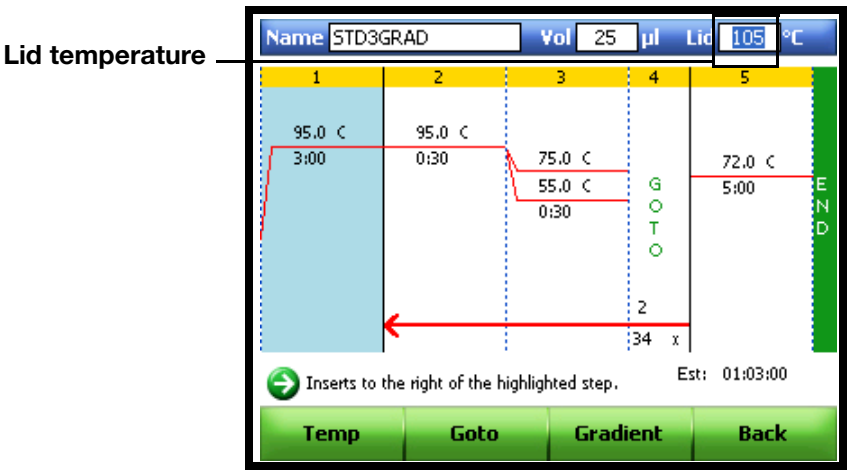
   TIP: Entering a sample volume between 1 and 50 selects Calculated temperature control mode, which is the standard mode. Entering zero (`0`) selects Block mode. Calculated mode is the recommended mode because it most accurately represents the actual sample temperature.

3. **Select the lid temperature**

   To change the default lid temperature for this protocol, select the lid temperature box (`Lid`) by pressing the arrow keys.

   NOTE: Heating the lid prevents condensation in the reaction vessels.

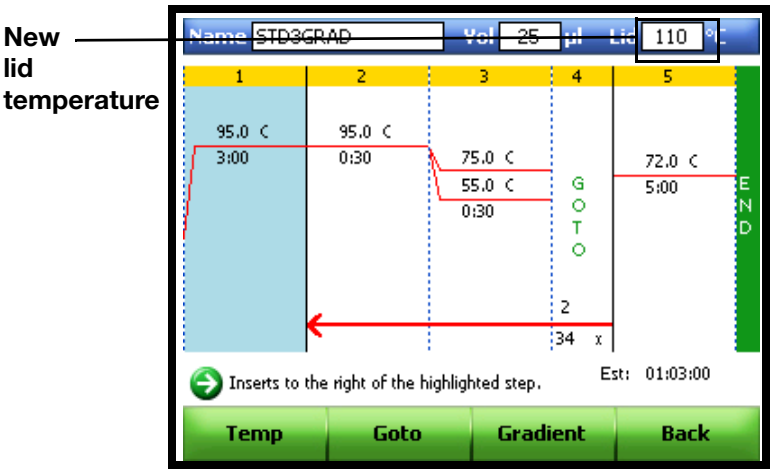
In this example, the default lid temperature is `105`°C:



4. **Enter the lid temperature**

   After selecting the `Lid` field, press the alphanumeric keys to enter a lid temperature.

   In this example, the new lid temperature is `110`°C:

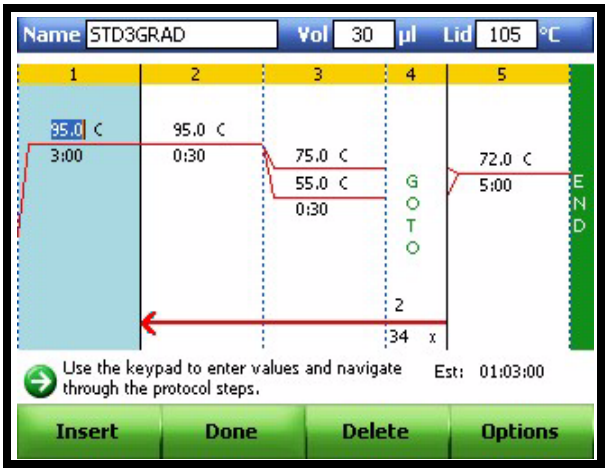


Saving a Protocol

To save a new protocol, you must enter a name (page 62). Once the protocol has a name, save it by following these instructions:

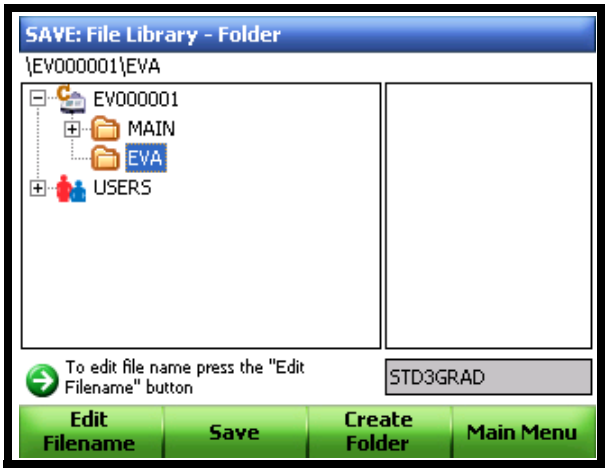
1. **Select** `Save` **to finish editing the protocol and save it**

   Once the protocol is completed, press `Done`**:**



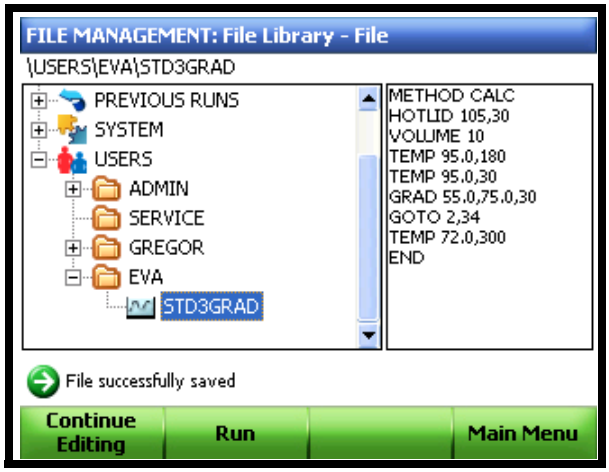
2. **Select a folder and press** `Save`

   Use the arrow keys to select the folder. In this example, the `EVA` **folder is selected:**



TIP: To change the name of the protocol, click `Edit Filename` **and type the new name. If you do not have a folder yet, click** `Create Folder` **to create a new folder.**

In this example, the file STD3GRAD is stored in the EVA folder:



3. **Select a new function (optional)**

   Once the protocol is saved in a folder, there are three options:
   - **Continue making changes to the protocol by clicking** `Continue Editing`
   - **Run the protocol by clicking** `Run` **(page 33)**
   - **Return to the main menu by clicking** `Main Menu` **(page 16)**

Saving an Edited Protocol

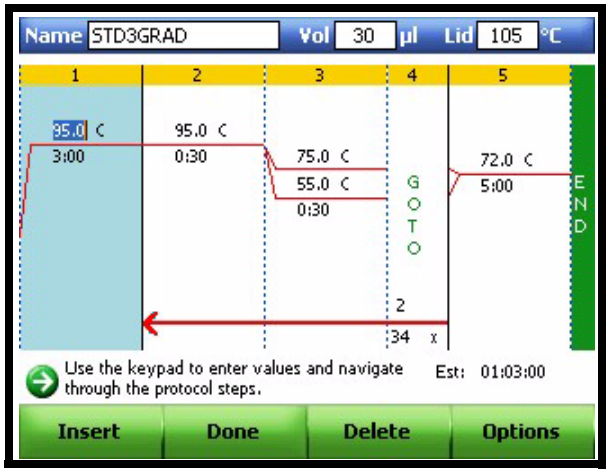
When editing an existing protocol, there are two options for saving it:

- **Replace the original protocol by saving the new protocol with the same name**
- **Save the protocol with a new name, and keep the original protocol intact**

To save an existing protocol, follow these steps:

1. **Press** `Save`

   Press the `Save` **button to begin the save process:**

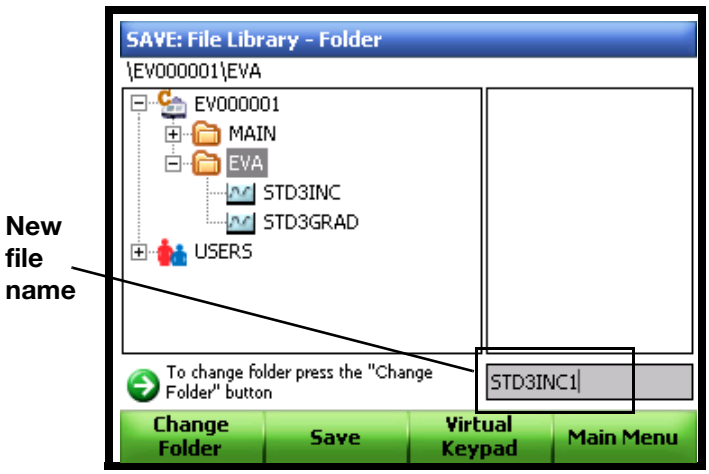


2. **Enter a new name (optional)**

   To save the protocol with a new name, select `Edit Filename` and type a new name.

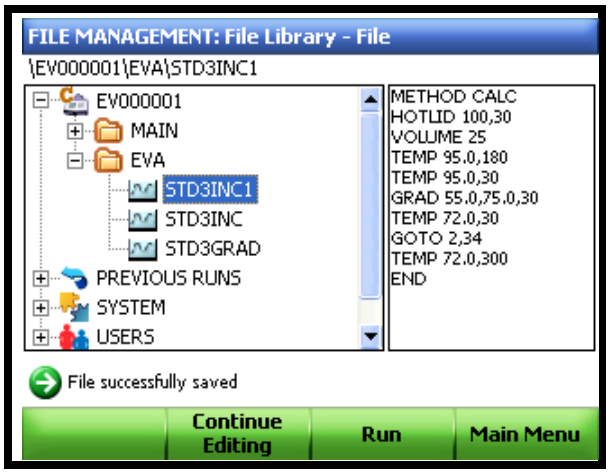
   NOTE: Saving the protocol with a new name adds a new protocol, and does not remove the original protocol or its name.

   In this example, the new name is STD3INC1:



3. **Select a folder**

   Notice that the C1000 thermal cycler opens the folder that contains the original protocol. In this example the edited file is named STD3INC:



   TIP: To keep the original file, rename the edited file with a different name.

4. **Choose `Save` to save the edited protocol.**

   Press the `Save` button to finish saving the edited protocol, and replace the original file.

Choosing the Sample Volume and Lid Temperature

Both sample volume and lid temperature influence the outcome of PCR:

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

The C1000 provides three ways of managing and entering these critical parameters:

- **Change the default setting in a protocol**
- **Change the default system settings in the** `Utilities` **(page 101)**
- **Change the setting when you run the protocol (page 33)**

Temperature Control Mode

The C1000 thermal cycler uses one of two temperature control modes to determine when the sample reaches the target temperature:

- **Calculated mode: When you enter a sample volume between 1 and 50 uL (or 30 uL for 384-well reaction modules), the thermal cycler calculates the sample temperature based on the sample volume. This mode is the recommended temperature control mode, because it most accurately represents the actual sample temperature.**
- **Block mode: When you enter a sample volume of zero (0) uL, the thermal cycler assumes the sample temperature is the same as the measured block temperature.**

Choosing a Lid Temperature

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

When running, the C1000's heated lid maintains the lid 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 thermal cycler's default lid temperature is 105°C.

> **NOTE: When the block is running an infinite hold at a temperature below the** `Cutoff` **parameter, the lid heater maintains 31.0°C. The default** `Cutoff` **setting is 30.0°C. To change the default** `Cutoff`**, select** `Utilities`**>** `Administrator Settings`**>** `Incubate Settings`**, while logged in as an Administrator.**

7 Optimize PCR With the C1000™

Optimizing PCR is critical for successful and repeatable reactions. Optimizing involves choosing the best reagents, as well as choosing optimal temperatures and hold times for each step of the protocol.

> **TIP: Use the Protocol Autowriter to generate a suggested PCR protocol to start with ("Creating a Protocol With the Protocol Autowriter" on page 24).**

This chapter describes how to use the features of the C1000 thermal cycler to further optimize PCR protocols:

- **Optimize for a faster PCR protocol (below)**
- **Optimize annealing temperature by including a temperature gradient (page 73)**
- **Optimize runs with small sample volumes (page 74)**
- **Transfer protocols that were run on another thermal cycler (page 75)**
- **Troubleshoot PCR problems (page 75)**
- **Select compatible PCR supplies, including microplates, sealers, tubes, and caps (page 76)**

For more extensive information and tutorials about optimizing PCR, refer to the Genomics web site (www.bio-rad.com/genomics). This site includes *Amplification Central* **where you can links to many resources for PCR experiments, including** *Tutorials*, *Assay Design* **tips, the** *PCR Doctor* **for troubleshooting and technical support. To open** *Amplification Central*, **log on to the Genomics web site (www.bio-rad.com/genomics), and select** *Support* **>** *Amplification Central.*

Optimize for Faster Protocols

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. Furthermore, optimizing the protocol for speed can also result in better PCR results.

> **TIP: Automatically create a protocol that is optimized for speed using the Protocol Autowriter (page 23).**

Complete optimization of a protocol involves selecting appropriate reagents, enzymes, and primers, as well as testing the parameters of the PCR protocol. For more detailed information about optimizing protocols for fast PCR, search the sites listed here.

These articles include tips for optimizing the reagents, in addition to the tips for optimizing a PCR protocol presented in this section:

- **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* **(page 3) for "Fast PCR".**

To optimize a PCR protocol to run faster using the features of the C1000 thermal cyclers, follow these guidelines:

- **Shorten the denaturing step during cycling**

  The initial denaturation step requires a longer hold time than denaturing steps during each 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 second for PCR products that are less than 500 base pairs (bp). Then test this shorter hold time to verify that a 1 second denaturation is sufficient to produce amplicons. Alternatively, add an increment (page 51) to the denaturation step to test for the best hold time.

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

  Most polymerases remain active throughout the typical range of annealing temperatures (55 to 70°C). Reduce the total run time by creating a two-step protocol that combines the annealing and extension steps in a single step. A two-step protocol can produce a yield that is similar to 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 your amplicon. Start with a hold time that is 10 seconds per 100 base pairs 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 "Optimize Annealing Temperature Steps With a Gradient" (page 73) for 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 will result in lower PCR yield, primer mismatches, 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 temperature gradient (page 50). 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 "Optimize Annealing Temperature Steps With a Gradient" (page 73) for 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 when you need a high percentage of complete amplicons at the end of the PCR. During each cycle, the extension step is typically 30 seconds. If amplification for 30 seconds is sufficient during cycling, then a longer final extension step is unnecessary.

To minimize the final extension step, choose a hold time between 30 seconds and 2 minutes for targets between 100 and 1,000 bp. Then test the hold time you choose 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 you adjust the number of cycles, you must know the approximate concentration of PCR template.**

  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, then shorten the number of cycles by 5 and 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.

Optimize Annealing Temperature Steps With a Gradient

For an efficient reaction and clean product, optimizing the annealing step is critical. 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.**

   Use the C1000's T_a Calculator 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.**

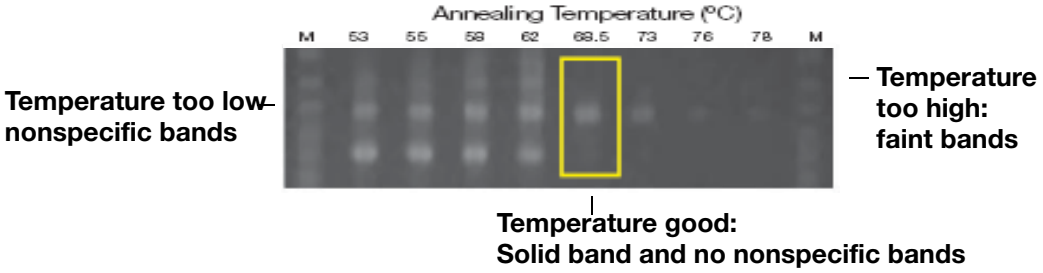
   Choose a gradient range that is as wide as possible to test for the optimal annealing temperature, and bracket the calculated T_a by 5 to 12°C. For example, if the calculated T_a is 55°C, then use a 24°C gradient to bracket that T_a. In this example, the gradient temperature range is from 43 to 67°C.

   NOTE: The widest possible gradient on the C1000 is 24°C.

3. **Choose a high annealing temperature from the results of the gradient PCR run.**

   By choosing a high annealing temperature, you reduce the chance of nonspecific primer binding. Select a high annealing temperature from the results of a PCR experiment with a gradient in the run. See step 2, above, for details

about creating a gradient. In this example, the gradient ran from 53 to 78°C. The best annealing is found at 68.5°C:



As a starting place, use the highest annealing temperature and subtract 1 or 2°C. Don't 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 PCR run to PCR run.

4. Narrow the temperature gradient in the annealing step (optional).

   Run a narrower annealing temperature gradient using the results from the first, wider temperature gradient. Bracket the annealing temperature that you choose in Step 3 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 from 55 to 65°C. Choose the final annealing temperature from a high successful temperature in the gradient experiment (as described in Step 3 on page 73).

Optimizing Runs With Small Sample Volumes

Running a protocol with a small volume requires optimization to prevent evaporation and condensation. Follow these suggestions for optimizing PCR with a sample volume below 10 uL. Read the following list for steps optimizing reactions with small sample volume:

- Control evaporation with a wax seal

  A wax such as Chill-Out®, is the best seal for reducing evaporation. Further reduction is possible by adding a second seal, such as a cap or film, in addition to the wax.

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

  When running an established protocol with a smaller sample volume (<10 uL) you might need to reoptimize the annealing temperature. If you observe mispriming or low amplification that is not a result of reagent problems, then adjust the protocol by optimizing the annealing temperature step using a gradient (page 73).

- 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 when the lid heat is the same. 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 volume, and therefore this is the best block for this application.

Transfer Protocols From Another Thermal Cycler

To transfer a PCR protocol from another thermal cycler to the C1000 and achieve the same results, you might need to lower the ramp rate. If the results of identical reactions run on a thermal cycler with a slower ramp rate do not result in the same data, then change the ramp rate.

Follow these suggestions when transferring a protocol:

- **Match the ramp rate of the other thermal cycler by changing the rate in each relevant step (page 53). In general, the ramp rate for the annealing step could be lowered when you move a protocol from a thermal cycler with a slower ramp rate to the C1000 thermal cycler**
- **Increase the amount of Mg^{++} in the reagents to help the primers anneal when the conditions have changed. This increase could cause some secondary PCR products, but should also increase the primary product**
- **Adjust the temperature in each step to reoptimize the protocol, and, most importantly, reoptimize the annealing temperature (page 73)**

Troubleshooting PCR Reactions

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

Follow these suggestions to reoptimize a reaction that fails by adjusting the protocol:

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

  Nonspecific products result from mispriming. When mispriming is not the result of a reagent problem, then adjust the annealing temperature.

  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. When the mispriming is not a result of a reagent problem, then adjust the hold time.

  Increase the hold-time in the annealing and extension steps to increase specificity of primer binding and provide 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 110) to increase amplification of the target product. Alternatively, decrease the annealing temperature, or run a gradient to optimize the annealing temperature (page 73).

Microplate, Tube, Sealer, and Cap Supplies

The 1000 series thermal cyclers run a large variety of standard supplies, such as microplates, tubes, sealers and caps. The supplies listed in this section are a subset of supplies that are verified by testing. They function within the specifications of the 1000 series thermal cyclers. However, these thermal cyclers are designed to work well with a broad range of standard supplies.

> **TIP: The composition and thickness of these supplies influences the outcome of a reaction. Whenever you significantly change the source or composition of supplies, it is good practice to reoptimize the protocol before you run an important experiment.**

Microplates, tubes, sealers, and caps come in a variety of compositions and colors. Bio-Rad tests the standard supplies listed in Table 10 for compatibility with the 1000 series thermal cyclers. For a full list of available reagents and supplies, refer to the Life Science Research Product catalog, which is available in print by contacting your local Bio-Rad Laboratories office (page 3), or online at discover.bio-rad.com.

> **NOTE: Sealing wax, such as Chill-out™ Liquid Wax is specifically recommended to seal small sample volumes of less than 10 uL. 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 Runs With Small Sample Volumes" (page 68).**

Table 10 lists the tested sealing options for microplates and tubes offered by Bio-Rad. However, if you have a verified source of these supplies, the C1000 thermal cycler is compatible with a wide variety of PCR supplies. Find a full list of supplies for PCR in any of these locations:

- **Life Science Research product catalog: Contact Bio-Rad for information about how to order a copy**
- **Life Science Research pages (discover.bio-rad.com): Go to the website and select** *Catalog Index* **>** *Amplification***.**
- **Go to the Gene Expression Gateway (www.bio-rad.com/genomics): Go to the website and select** *Products* **>** *Amplification***.**

In Table 10, each series of supplies lists with the prefix of the catalog numbers:

Table 10. Compatible sealing options for PCR microplates and tubes.

TUBES AND MICROPLATES		CAPS AND SEALERS	
Description (Catalog Prefix*)	Volume and Number	Description (Catalog Prefix*)	
Tubes			Caps (TCS*)
Individual tubes with caps (TFI* and TWI*)	0.2 ml		(Included)
Strip tubes (TBS*)	0.2 ml, 8- and 12-tube		Strip
Strip tubes, low profile (TLS*)	0.2 ml, 8-tube		Strip
Microplates		Film (MS*)	Caps (TCS*)
Unskirted and full height (MLP*)	24-, 48-, and 96-well	A, B, F	Strip
Unskirted and low profile (MLL*)	48- and 96-well	A, B, F, P	Strip

Table 10. Compatible sealing options for PCR microplates and tubes.

TUBES AND MICROPLATES		CAPS AND SEALERS	
Skirted and full height (MSP*)	96- and 384-well	A, B, F	Strip
Skirted, full height, and hard-shell (HSP*)	96- and 384-well	A, B, F	Strip

*** The prefix of the catalog number for each series of supplies.**

8 Protocol Files and Folders

Read this chapter to learn about the components of a C1000 protocol, and how to manage protocol files:

- Protocol steps and options (below)
- Managing protocol files and folders, including copying, renaming, deleting, and moving files and folders (page 82)

Protocol File Content

A PCR protocol is based on temperature steps, where the sample is kept at a target temperature for a specific amount of time (called the hold time). A PCR protocol also includes a cycle, where temperature steps repeat many times. A gradient temperature step is a specialized step that includes a temperature gradient across the block.

In general, a PCR protocol includes these types of steps:

1. An initial temperature step

   This initial temperature step denatures the template, and also activates the polymerase.

   To add an initial step in the C1000 protocol file, add a temperature step (page 48).

2. A cycle of steps to amplify the PCR product

   This set of temperature steps repeats in a cycle and amplifies the PCR product. Typically the cycle includes three temperature steps called denaturing, annealing, and extension steps. These steps denature the DNA template, anneal the primer to the template, and allow the polymerase to extend the complementary strand of DNA. However, under certain conditions the annealing and extension steps combine in a shorter two-step cycle.

   To add the denaturing, annealing, or extension steps in the C1000 protocol file, add a temperature step (page 59) or gradient step (page 50). To cycle these temperature steps, add a GOTO step immediately after the last step that you want to cycle (page 57).

3. A final temperature step to extend the PCR product

   This final extension temperature step serves to finish extending any PCR product that did not fully elongate during cycling.

   To add a final extension step in the C1000 protocol file, add a temperature step.

The C1000 protocol step varies from the typical PCR protocol steps. When you transfer a typical PCR protocol to the C1000, you translate the steps into the three types of protocol steps:

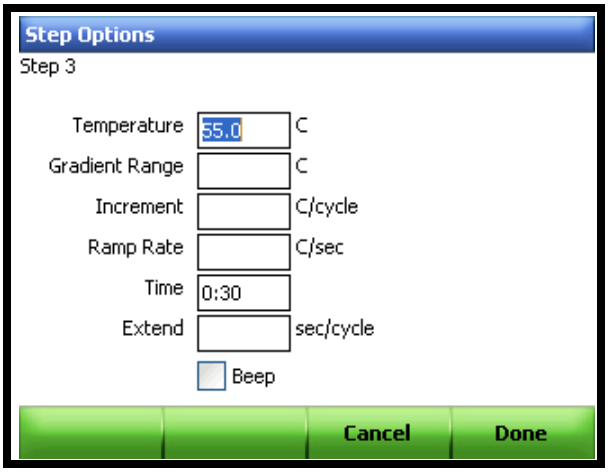
- **Temperature, which is the same as a typical PCR protocol step**
- **Gradient, which is a temperature step that includes a thermal gradient**
- **GOTO, which is used to add loops and create cycle in PCR**

C1000 thermal cycler protocol steps and parameters.

Step Name	Parameters and Ranges	Description
Temperature	Temperature °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**
	Time: **The hold time between 1 second and 18 hours in the format of hours:minutes:seconds. To enter an infinite hold, press the ∞ (Infinite, 0) key**	
Gradient	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.** **Range: The gradient range**	**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 second and 18 hours in the format of hours:minutes:seconds. To enter an infinite hold, press the ∞ key (zero, 0) key**	
GOTO	GOTO**: The 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 temperature steps loop**	

Options for Temperature and Gradient Steps

Each C1000 temperature or gradient step can include additional parameters. The Options screen displays these parameters. The following shows the Options screen:



Each temperature step can include these parameters:

- **Temperature and Time (required): Change target temperature and the hold time in a step**
- **Gradient: Create a temperature gradient across the block during the step**
- **Increment: Change the target temperature with each cycle**
- **Ramp Rate: Change the ramp rate for the step**
- **Extend: Extend the hold time in the step with each cycle**
- **Beep: Add a beep at the end of the step**

Table 9 describes all the optional parameters for temperature and gradient steps. The table also includes the limits and range of the parameters:

Table 11. Options for temperature and gradient steps

Option	Parameter and Range	Description
`Increment`	**A temperature from -10.0 to 10.0°C per cycle in tenths of a degree**	**Applies only to a temperature step (page 48). 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**
`Ramp Rate`	**A number from 0.1 to 5°C/ second.**	**Applies only to a temperature step (page 48). Instructs the thermal cycler to ramp to the target temperature at the specified ramp rate in that step. Applies only to the Dual 48 and 96 Fast reaction modules**
`Extend`	**A time from -60 to 60 seconds per cycle**	**Applies to both temperature and gradient steps. Instructs the thermal cycler to extend the hold time with each cycle, where a positive number increases the hold time, and a negative number decreases the hold time**
`Beep`	**(No parameters)**	**Applies only to a temperature step (page 48). Instructs the thermal cycler to beep to signal that the thermal cycler reached the target temperature of that step**

For more instructions about creating a protocol with these steps, see page 47.

Managing Protocol Files and Folders

To manage protocol files and folders, press the `FILES` button from the `Main Menu` to open the file library. The menu of buttons in the file library provides options for managing files and folders. The menu button options will change based on what is selected in the file library. The below table lists all the functions in the Files option:

Table 12. List of functions in the `FILES` option

Function	Description
PROTOCOLS:	
`Copy/Move Protocol`	Copies or Moves an existing protocol to another folder location
`Delete Protocol`	Deletes protocol
`Rename Protocol`	Renames protocol
FOLDERS:	
`Create Folder`	Creates a new folder
`Copy All`	Copy all files from one destination to another existing protocol folder
`Copy/Move Folder`	Copies or Moves an existing folder to another cycler or thumbdrive
`Delete Folder`	Deletes folder and all contents
`Rename Folder`	Renames folder
`Rename Cycler`	Renames thermal cycler
`Main Menu`	Returns to Main Menu

NOTE: File names can have a maximum of 8 characters

Creating and Copying Folders, Renaming Thermal Cyclers

The File Library, at the unit level, displays the functions available for managing folders and renaming thermal cyclers. This section provides step-by-step instructions for using those functions:

- **Select `Create Folder` to create a new folder on the C1000 thermal cycler, an attached S1000 or a thumbdrive.**
- **Select `Copy All` to copy all files from one source to another attached cycler or USB thumbdrive.**
- **Select `Rename Cycler` to rename your thermal cycler**
- **Select `Main Menu` to return to the Main Menu**

Create a Folder

To begin creating a new folder, select `FILES` from the Main Menu, then select `Create Folder`.

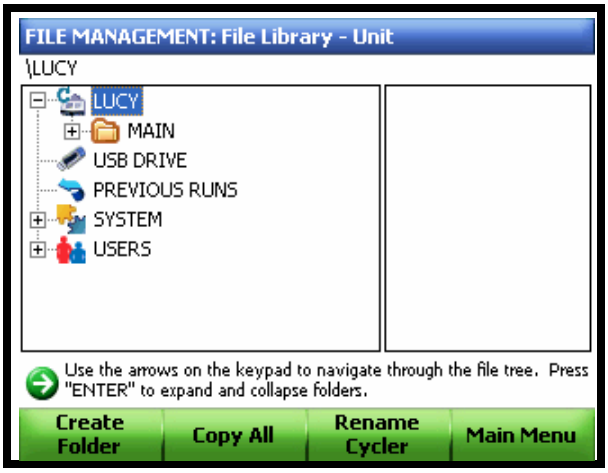
To create a new folder, follow these instructions:

1. **Select the cycler or thumbdrive to create a new folder**

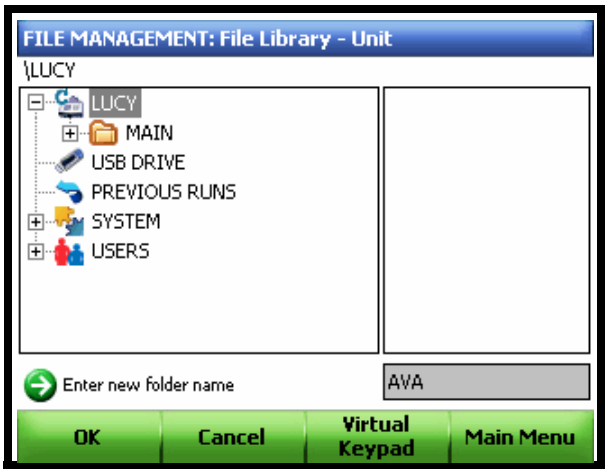
2. **Select** `Create Folder`
3. **Enter a folder name, press** `ENTER`

In this example, the cycler `LUCY` **is selected and a new folder,** `AVA` **will be created:**

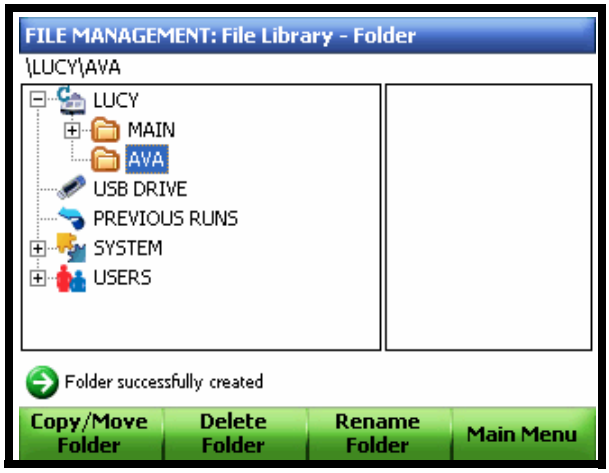
- **The cycler** `LUCY` **is selected, press** `Create Folder` **(**`F1`**)**



- **Enter the folder name in the text box, press** `OK`



- **Confirmation that folder was created successfully**



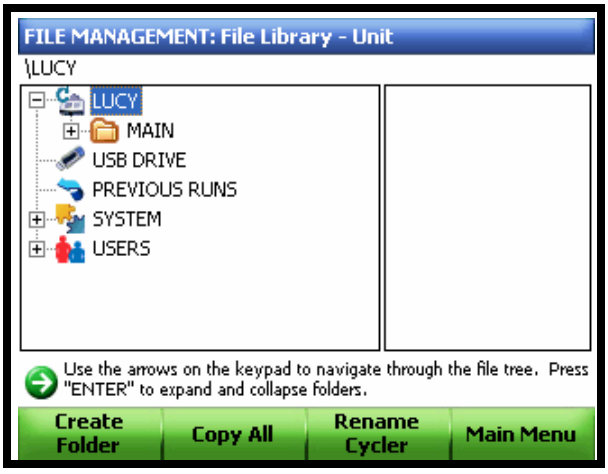
Copy All

To copy all files from one destination to another, select `FILES` **from the Main Menu, then select** `Copy All`**.**

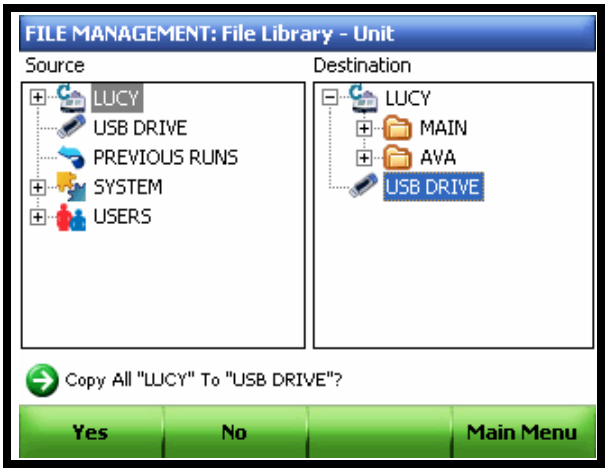
To `Copy All`**, follow these directions:**

1. **Select the cycler or thumbdrive to** `Copy All`
2. **Select** `Copy All`**, destination choices will appear in the right side window**
3. **Using the** `UP` **&** `DOWN ARROWS`**, select the destination to copy all, press** `ENTER`
4. **Press** `YES` **to confirm**

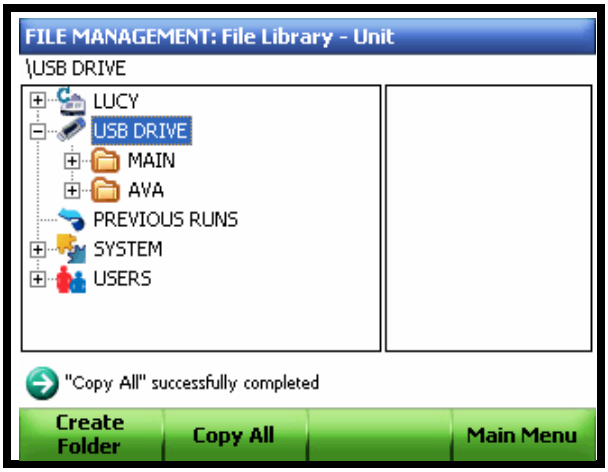
   In this example, all files under `LUCY` **were copied to the USB Drive**
   - **Cycler** `LUCY` **is selected, press** `Copy All` **(**`F2`**)**



- **Choose destination to copy all contents of Lucy, press** YES



- **Confirmation that copy all was successful**



Rename Cycler

To rename the thermal cycler, select FILES **from the Main Menu, then select** Rename Cycler**.**

> **NOTE: You must be logged in as an administrator to rename the thermal cycler.**

To rename the cycler, follow these directions:

1. **Select** Rename Cycle
2. **Change the cycler name in the text box, press** OK

   NOTE: File names can have a maximum of 8 characters

Copying, Moving, Deleting, and Renaming Folders

The file library- folder level, displays the functions available for managing folders. This section provides step-by-step instructions for using those functions:

- **Select** `Copy/Move Folder` **to create or move a folder on the C1000 thermal cycler, an attached thermal cycler or a thumbdrive**
- **Select** `Delete Folder` **to delete a folder to copy all files from one thermal cycler to an attached cycler or USB thumbdrive**
- **Select** `Rename Folder` **to rename an existing folder**
- **Select** `Main Menu` **to return to the Main Menu**

Move a Folder

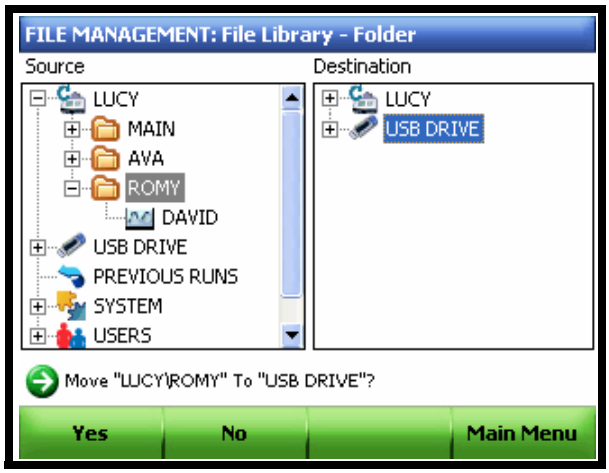
To begin copying or moving a folder, select `FILES` **from the Main Menu, then use the** `ARROWS` **to select a folder, then press** `Copy/Move Folder`**.**

To create a new folder, follow these instructions:

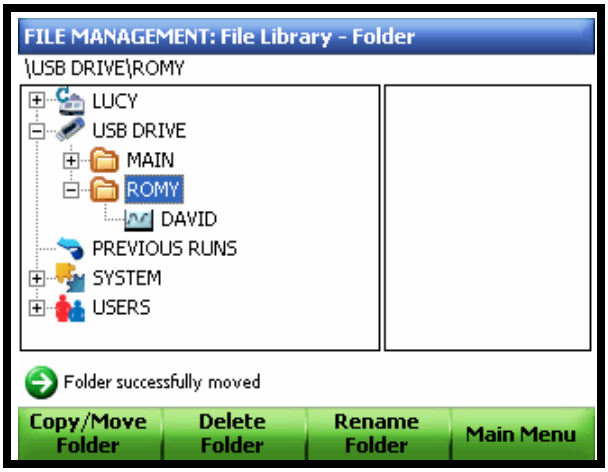
1. **Select** `Move Folder`
2. **Using the** `UP` **&** `DOWN ARROWS`**, select new destination for folder**
3. **Press** `YES` **to confirm**

   NOTE: The `MAIN` **folder cannot be moved**

   In this example, the folder `ROMY` **is moved from cycler** `LUCY` **onto the USB key**
   - **Select the** `ROMY` **folder under the** `LUCY` **cycler, press** `Copy/Move Folder` **(**`F1`**)**
   - **In the right hand window, select the USB drive to move the ROMY folder to the USB Drive, Press** `YES` **(**`F1`**)**



- **Confirmation that folder was successfully moved**



Copy a Folder

To begin copying a folder, select `FILES` **from the Main Menu, then use the** `ARROWS` **to select a folder, then press** `Copy/Move Folder`**.**

To copy a folder, follow these instructions:

1. **Select** `Copy Folder`
2. **Using the** `UP` **&** `DOWN ARROWS`**, select new destination for folders**
3. **Press** `YES` **to confirm**

Delete a Folder

To begin deleting a folder, select `FILES` **from the Main Menu, then use the** `ARROWS` **to select a folder, then press** `Delete Folder`**.**

To delete a new folder, follow these instructions:

1. **Using the** `UP` **&** `DOWN ARROWS`**, select folder to delete**
2. **Select** `Delete Folder`
3. **Press** `YES` **to confirm**
   NOTE: The `Main` **Folder cannot be deleted**

Rename a Folder

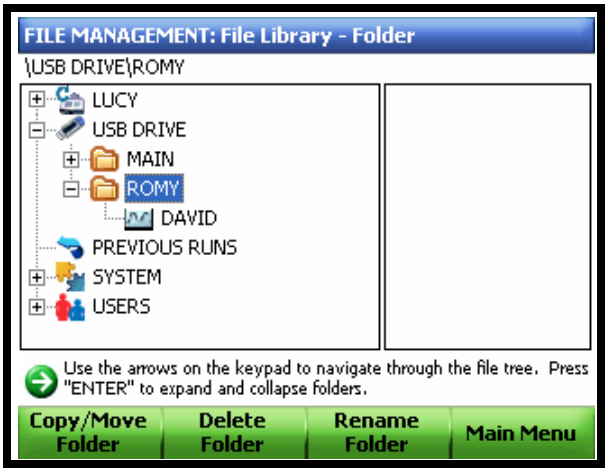
To begin renaming a folder, select `FILES` **from the Main Menu, then use the** `ARROWS` **to select a folder, then press** `Rename Folder`**.**

To rename a folder, follow these instructions:

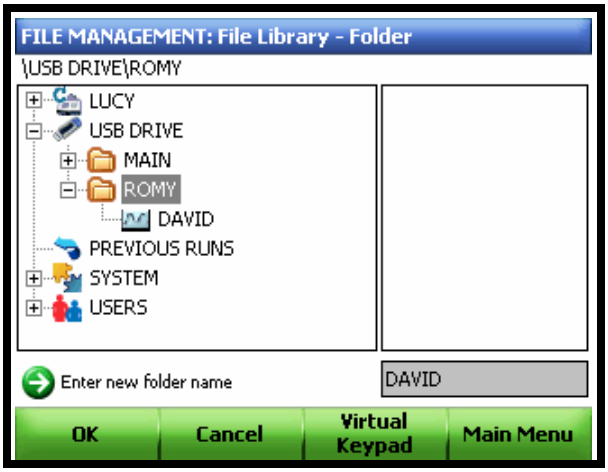
1. **Select** `Rename Folder`
2. **Enter a new name in the text box, press** `OK`
   NOTE: The `MAIN` **Folder cannot be renamed**

In this example, the folder `ROMY` is renamed to `DAVID`

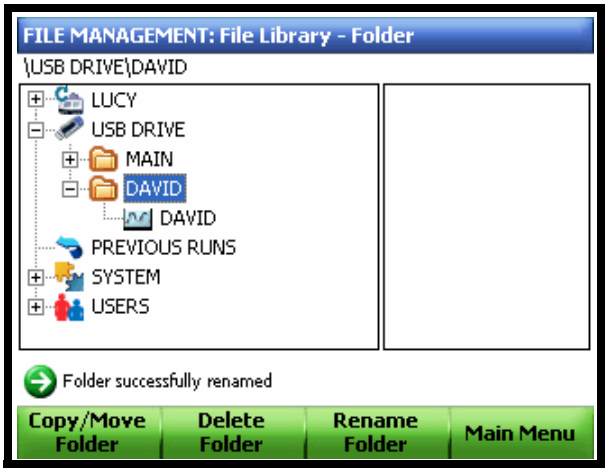
- **Highlight Folder to rename**



- **Enter new Folder name**



- **Confirmation that folder was successfully renamed**



Copying, Moving, Deleting, and Renaming Protocols

The File Library, at the file level, displays the functions available for managing protocols. This section provides step-by-step instructions for using those functions:

- **Select** `Copy/Move Protocol` **to copy or move a protocol on the C1000 thermal cycler, an attached thermal cycler or a thumbdrive.**
- **Select** `Delete Protocol` **to delete a protocol to copy all files from one thermal cycler to an attached cycler or USB thumbdrive.**
- **Select** `Rename Protocol` **to rename an existing protocol**
- **Select** `Main Menu` **to return to the Main Menu**

Copy a Protocol

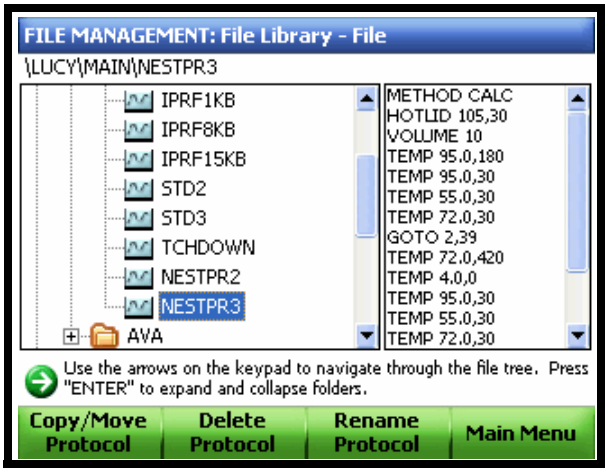
To begin copying a protocol, select `FILES` **from the Main Menu, then use the** `ARROWS` **to select a protocol, then press** `Copy/Move Protocol`

To copy a protocol, follow these instructions:

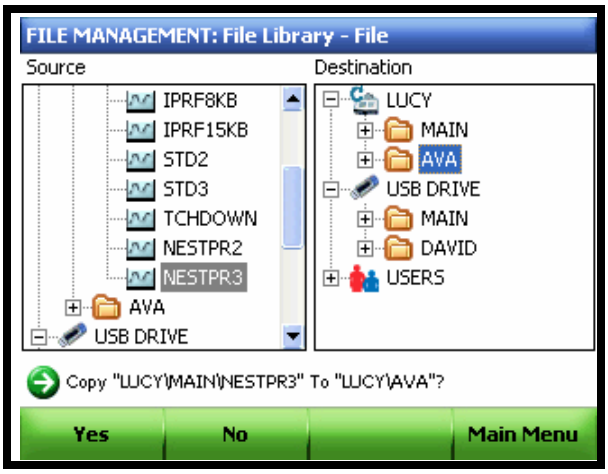
1. **Select** `Copy Protocol`
2. **Using the** `UP & DOWN ARROWS`**, select folder destination**
3. **Press** `YES` **to confirm**

In this example, the protocol `NESTPR3` **is copied into folder** `AVA`

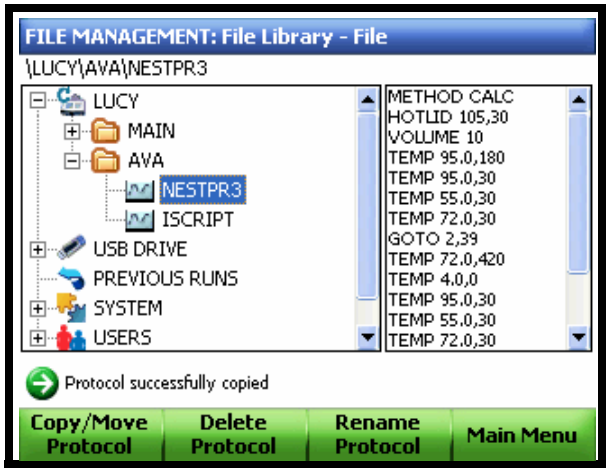
- **Select Protocol to copy, press** `Copy/Move Protocol` **(**`F1`**)**



- **Select Folder to copy protocol into, Press** YES



- **Confirmation that protocol was successfully copied**



Move a Protocol

To begin moving a protocol, select FILES **from the Main Menu, then use the** ARROWS **to select a protocol, then press** Copy/Move Protocol

To move a protocol, follow these instructions:

1. **Select** Move Protocol
2. **Using the** UP & DOWN ARROWS**, select new folder destination**
3. **Press** YES **to confirm**

Delete a Protocol

To begin deleting a protocol, select FILES **from the Main Menu, then use the** ARROWS **to select a protocol, then press** Delete Protocol

To delete a protocol, follow these instructions:

1. **Using the** UP & DOWN ARROWS**, select folder to delete**
2. **Select** Delete Protocol

3. **Press** `Yes` **to confirm**

9 Maintenance and Troubleshooting

This chapter includes suggestions for maintaining and troubleshooting the C1000™ thermal cycler.

- Clean and maintain the C1000 thermal cycler for optimal safety and performance (below)
- Maintain sufficient air flow around the thermal cycler to optimize thermal control while running PCR (page 95)
- Troubleshoot thermal cycler error messages, including how to interpret and respond to error messages (page 96)

Cleaning and Maintaining the C1000 Thermal Cycler

The C1000 thermal cycler requires little maintenance to maintain proper operation and precise thermal control. However, with long and constant use, the thermal cycler will require some cleaning and other maintenance:

- Clean the base (below)
- Clean the reaction module (page 94)
- Replace the fuses (page 96)

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

Cleaning the C1000 Thermal Cycler Base

The C1000 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. In general, always use a soft cloth and water to wipe off spilled solutions and debris immediately.

> 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 base, follow these instructions, paying careful attention to the warnings:

WARNING! 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 this panel prevents damage that will obscure the display.**

  NOTE: Use of abrasive detergents or rough material will scratch the control panel.
- **Clean the reaction module bay: Clean with a damp soft cloth to remove debris and spilled liquids. Cleaning this 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. Scratches and damage to this surface interfere 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 base cover: Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution. Cleaning the cover prevents corrosion.**

Cleaning the Reaction Modules

Clean the C1000 reaction modules on a regular schedule to prevent reagents from accumulating and interfering with the ability of the block to change temperature quickly.

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

WARNING! Prevent electrical shock. Always remove the reaction module from the thermal cycler base before cleaning it.

- **Clean the cooling fins: Remove dust with a soft brush or damp cloth. Remove any heavy dust that is deep in the vents 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 residue completely. Cleaning the fins improves precise sample heating and cooling.**

  WARNING! Clean the block: Use a soft cloth and water to remove debris from the outer block. Never use abrasive detergents, caustic solutions, or rough material that will scratch the 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. Use disposable plastic pipettes 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! Bleach, ethanol, or soap that is left in the blocks 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.

Use of oil in the wells is not recommended. However, if oil is used, the wells must be cleaned thoroughly and often. 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 will damage the block, lid, and thermal cycler base.

- **Clean the inner lid: Use a soft cloth and water to remove debris and solutions from the inner lid surface. Never use abrasive detergents or rough material that will scratch the surface. Cleaning the inner lid improves precise sample heating and cooling.**
- **Clean the outer lid surface: Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and then rinse the surface with a damp cloth. Cleaning the cover will prevent corrosion.**

Maintaining Sufficient air flow

The C1000 thermal cycler requires sufficient air flow to heat and cool precisely. 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 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 you first set up the C1000 thermal cycler in a new location, you can test for sufficient local air flow by following these steps:

1. **Set up the instrument where you plan to use it and turn on the power**

   For instructions about setting up the thermal cycler, see page 5.

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 minutes**

   To run a protocol you do not need to include a sample, but must include an empty microplate or tubes. The lid will 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, use the following list 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 a thermal cycler:

- Adjust air conditioning to lower the ambient air temperature
- Move the thermal cycler to another location
- Provide more space around the C1000 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

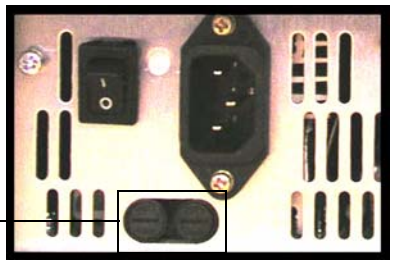
The C1000 thermal cycler fuses are designed to blow in case of severe power surges or other causes of electrical short. This process protects both user and instrument from excessive electric charge. These fuses rarely need to be replaced. However, some institutions prefer to replace fuses on a regular 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 (page 115). To replace a power cord, contact Bio-Rad Technical Support (page 3).

Finally, check that the fuses are intact. The C1000 runs with two fuses that have specifications listed in page 115. To remove and check the fuses, follow these steps:

WARNING! 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



2. Pull out the fuse drawer and remove the fuses
3. If the 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 Thermal Cycler Error Messages

In general, when the C1000 displays a warning or error message the instructions for fixing the problem are contained in the message. For example, the warning message that displays when external power fails during a run (page 97). This error message tells you what protocol was running, when the protocol stopped due to the power failure, and the block temperature when the thermal cycler resumed the run.

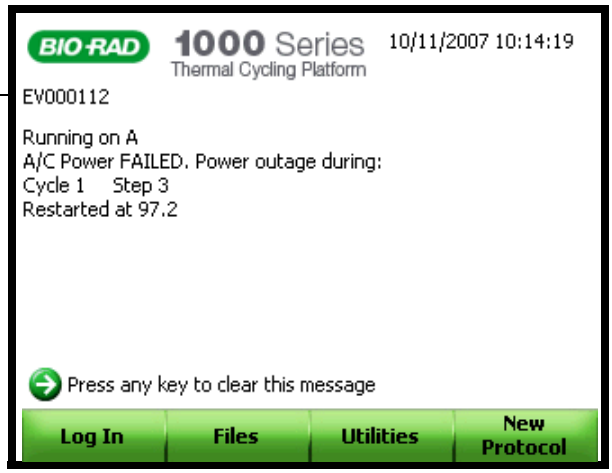
This section includes explanations for the following warnings and error messages

- **Restarting after a power failure (below)**
- **C1000 thermal cycler error messages (page 97)**

Restarting After a Power Failure

If the external power is interrupted when the thermal cycler is running a protocol, the C1000 resumes running the protocol as soon as power is restored. In this case, the following error message will alert you that the protocol was interrupted:

Protocol name



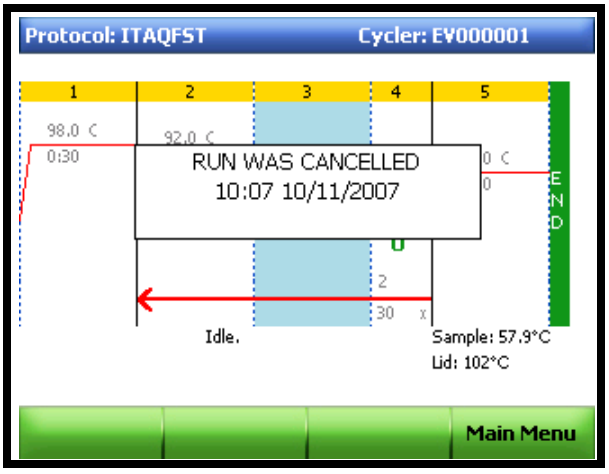
The power failure is also recorded in the `Run Logs`**.**

> **WARNING! 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.**

Warning and Error Messages

The C1000 tracks errors that occur during a run. After a run, all messages are displayed. For example, when a run is cancelled, the following `PROTOCOL CANCELLED` **screen is displayed:**

Protocol cancelled screen



Press the `ENTER` **key to clear a message or error, and continue to the next screen. When all messages are cleared, the C1000 returns to the** `Main menu`**.**

Error messages are recorded in the `Run Logs`**.**

> **TIP: The C1000 also tracks errors and system messages for all attached S1000 thermal cycler.**

Table 13. Warning and error message solutions.

Message	Cause
Running On A A/C Power Failed Power Outage During Cycle X Step Y Restarted At Zz.z Press Any Key To Clear This Message	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. Block overheated. Please call Bio-Rad for service.	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. Teosinte overheated. Please call Bio-Rad for service.	Teosinte 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.
All block sensors failed. Please call Bio-Rad for service.	All block sensors have failed (see below for failure criteria). Protocol terminated.
Power supply overheated. Please call Bio-Rad for service.	Power Supply temperature has exceeded 85°C. Protocol terminated.
Heated lid failed. Protocol cancelled. Please call Bio-Rad for service.	Lid sensor has failed during lid preheat. Protocol terminated.
Internal fan failed. Please call Bio-Rad for service.	<unused at present>
Heatsink overheating. Please check air flow. Please call Bio-Rad for service.	Heatsink has exceeded 70°C. System beeps and displays error.
Please check air flow. System overheating. Please call Bio-Rad for service.	Amp temp has exceeded 80°C. System beeps and displays error.
Please check air flow. Power supply overheating. Please call Bio-Rad for service.	Power Supply temperature has exceeded 80°C. System beeps and displays error.
Slow block cycling. Please call Bio-Rad for service.	Block failed to achieve target in the estimated time.
Slow lid cycling. Please call Bio-Rad for service.	Lid failed to achieve target in the estimated time.

Table 13. Warning and error message solutions.

Message	Cause
Slow gradient. Please call Bio-Rad for service.	**Block failed to achieve gradient in the estimated time.**
Heated lid failed. Please call Bio-Rad for service.	**(singles only) If the right and left lid heater channels deviate from each other by more than 5°C the lid is shut off.**
Block sensor 0 failed. Please call Bio-Rad for service.	**Block sensor 0 has failed* and the protocol was terminated.**
Block sensor 1 failed. Please call Bio-Rad for service.	**Block sensor 1 has failed* and the protocol was terminated.**
Block sensor 2 failed. Please call Bio-Rad for service.	**Block sensor 2 has failed* and the protocol was terminated.**
Block sensor 3 failed. Please call Bio-Rad for service.	**Block sensor 3 has failed* and the protocol was terminated.**
Block sensor 4 failed. Please call Bio-Rad for service.	**Block sensor 4 has failed* and the protocol was terminated.**
Block sensor 5 failed. Please call Bio-Rad for service.	**Block sensor 5 has failed* and the protocol was terminated.**
Left lid sensor failed. Please call Bio-Rad for service.	**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.**
Right lid sensor failed. Please call Bio-Rad for service.	**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.**
Left heatsink sensor failed. Please call Bio-Rad for service.	**Left heatsink sensor has failed*, system using average of amplifier temperatures to continue.**
Right heatsink sensor failed. Please call Bio-Rad for service.	**Right heatsink sensor has failed* system using average of amplifier temperatures to continue.**
Lid overheated and was shut off. Please call Bio-Rad for service.	**(duals only) Lid has overheated and has been shut off and protocol has been terminated.**
Amplified 1 temperature sensor failed. Please call Bio-Rad for service.	**Amplifier temperature sensor 1 has failed*.**
Power supply sensor failed. Please call Bio-Rad for service.	**Power Supply sensor has failed*.**
Block power failure. Protocol cancelled. Please call Bio-Rad for service.	**Power to block is out of range.**
Logic power failure. Please call Bio-Rad for service.	**Logic power sensor is out of bounds.**

Table 13. Warning and error message solutions.

Message	Cause
Base power failure. Protocol cancelled. Please call Bio-Rad for service.	Base power sensor is out of bounds. Protocol cancelled.
Amplifier 2 sensor failed. Please call Bio-Rad for service.	Amplifier temperature sensor 2 has failed*.
Corrupt protocol memory detected. Protocols may be lost. Please call Bio-Rad for service.	Protocol storage memory has been corrupted.
Unable to read reaction module header. Please restart cycler. 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 was shut off. Please call Bio-Rad for service.	There was a problem with the block and power was shut off
One of the block sensors in a single block has failed. The system has cancelled the protocol and sent the block to 4°C to preserve the samples. Please call Bio-Rad for service.	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 (page 3).

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

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.

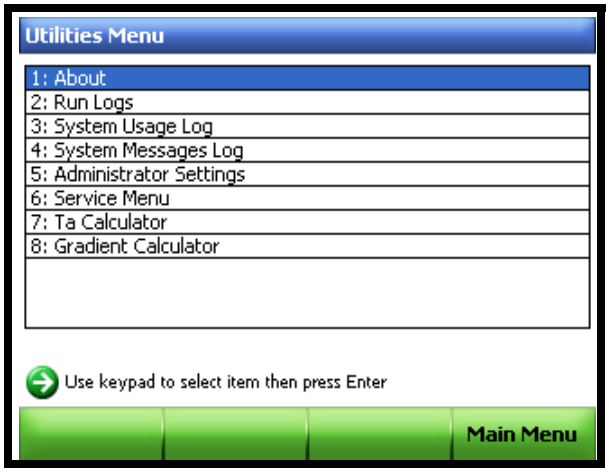
10 Advanced Functions

The C1000™ thermal cycler provides open or limited access to system and administrative functions. This chapter provides an overview of the advanced functions, including the Utilities menu, Administrator menu, System folder, and data ports.

- Utilities menus and functions (below)
- Administrator functions (page 102)
- Data in the SYSTEM folder (page 104)
- Connect USB devices to the C1000 through the data ports to connect devices, including how to connect a USB drive, mouse, or keyboard (page 104)
- Connect multiple S1000 thermal cyclers to the C1000 (page 105)
- Running the C1000 under robotic control in a high-throughput laboratory (page 107)

Utilities Menus and Functions

The Utilities menu provides a list of functions that control the C1000 thermal cycler.



The menus and functions in the Utilities offer the following options:

- `About:` **View the current firmware versions and serial numbers.**

- `Run Logs:` **View the run log for each time a protocol is run. Logs can also be copied onto a USB key (for use when servicing the cycler)**
- `System Usage Log:` **View a log that contains a list of all events that occurred during each run**
- `System Messages Log:` **View messages that occurred during all runs**
- `Administrator Settings:` **Open the Administrator functions (page 102)**

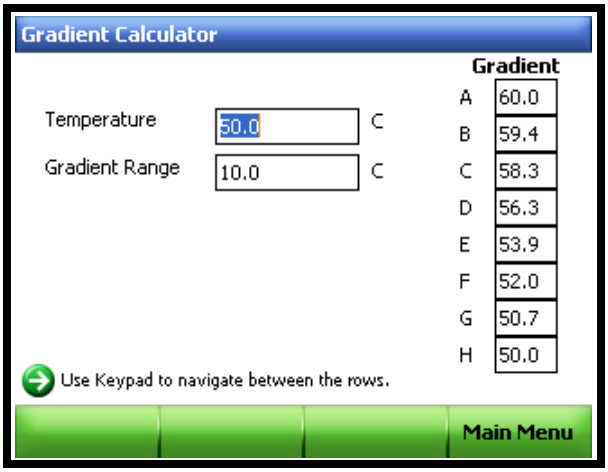
  NOTE: Only a logged in Administrator has access to Administrative settings.
- `Service Menu:` **Functions limited to service personnel from Bio-Rad Laboratories**
- `Ta Calculator:` **View and use the T_a calculator to calculate the melting temperature for a pair of primers**
- `Gradient Calculator:` **Calculate a temperature gradient across a block**

Gradient Calculator Function

The `Gradient Calculator` displays the thermal gradient across a block. Use this calculator to view the temperature in a specific well or row.

To calculate and view the thermal gradient on the block, follow these steps:

1. **Open the Utilities menu**
2. **Select `Gradient Calculator`**
3. **Enter the `Lower` temperature and `Gradient Range`:**

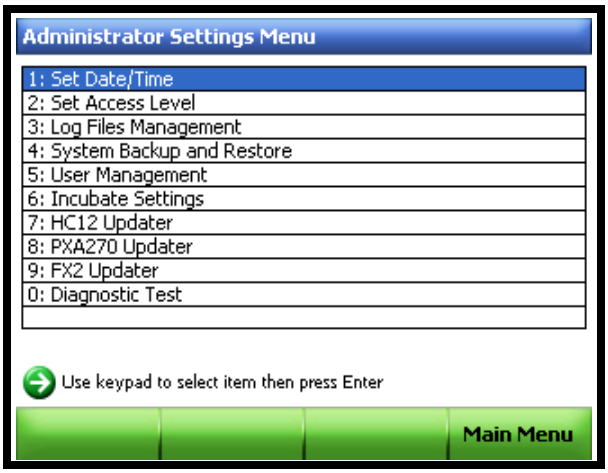


Administrator Settings Menu

NOTE: Only a logged in Administrator may access the `Administrator Settings` menu.

The `Administrator Settings` menu contains all the functions needed to set access levels and system defaults for the C1000 thermal cycler. Open this list by

selecting `Administrator Settings Menu` **in the Utilities list, and pressing** `ENTER`**. The image below shows options listed in the Administrator Settings Menu:**

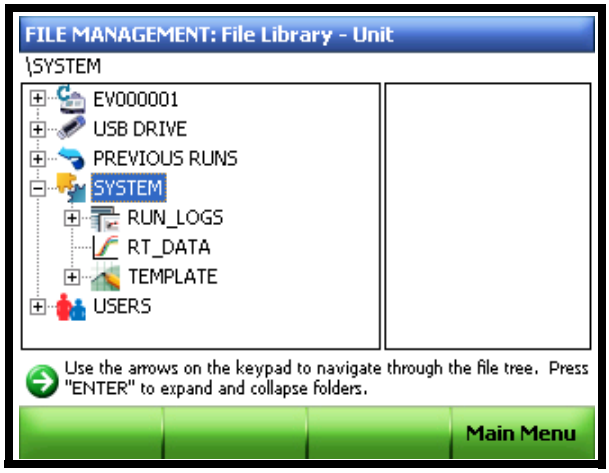


The Administrator Settings menu contains these options:

- `Set Date/Time`**: Select this option to set the date and time on the thermal cycler**
- `Set Access Level`**: Select this option to set the user access levels to** `Open` **or** `Secured`
- `Log Files Management`**: Select this option to clear or trim log files**
- `System Backup and Restore`**: Select this option to backup or restore system settings from a USB drive**
- `User Management`**: Select this option to add or remove users and clear passwords**
- `System Settings`**: Select this option to set the default incubate settings for the lid temperature, lid cut-off, and incubation temperature**
- `HC12 Updater, PXA270 Updater, FX2 Updater`**: Select these options to update the firmware on the thermal cycler**
- `Diagnostic Test`**: Select this option to perform a series or subset of diagnostic self tests on the cycler (for use when servicing the cycler)**

SYSTEM Folder Functions

The SYSTEM folder provides access to system-wide templates and data. The image below shows the content of the SYSTEM folder:



The folders within the SYSTEM folder provide access to these functions:

- `RT DATA:` **View real-time data that was collected with the CFX96 or CFX384 real-time detection instruments**
- `RUN LOGS`**: View or download the logs from a run]**
- `TEMPLATE:` **Edit or create the default protocol file for the thermal cycler**

Using Data Ports

The C1000 has several data ports that can be used to transfer data to and from the thermal cycler:

- **A USB A port below the control panel:**

  Use this port to transfer data to and from a USB drive, or to attach a USB mouse or keyboard.
- **A USB B computer port on the back panel:**

  Use this port to connect a computer to run the C1000 thermal cycler and any attached S1000 thermal cyclers.
- **Four USB A ports located on the back panel:**

  Use these ports to connect up to four S1000 thermal cyclers, USB drives, a mouse, or a keyboard.
- **A test port on the back panel**

Attaching USB B devices

Use USB B ports to connect the C1000 thermal cycler to a computer.

Attaching USB A devices

The USB A port can attach any of the following USB devices:

Connect a keyboard

Connect a USB drive

Connect a bar code reader

Connect up to three S1000 thermal cyclers

To connect the S1000 directly to a C1000 follow these instructions:

1. **Plug a USB cable into the S1000**

   Plug a high quality, shielded USB cable into the USB B port on the back of the S1000. For USB cable part numbers, see page 118.

2. **Plug the USB cable into the C1000**

   Plug the other side of the USB cable into a USB B port on the back of the C1000. The C1000 will detect the S1000.

3. **Open the main screen or instrument tree on the C1000**

   Open the main menu or instrument tree on the C1000, or in the 1000 series software if the C1000 is connected to a computer.

4. **Select the S1000**

   Select the S1000 by serial number or name. If the S1000 has a name, then the name will be displayed instead of the serial number.

Attaching to the Test Port

The serial port is used to test the thermal cycler by service personnel.

Control an S1000 With the C1000

Up to three S1000s can run under the control of a C1000. The S1000 can connect to the C1000 through a USB hub or in a "daisy chain" configuration, where the S1000 is connected, in series, to a C1000.

When connected to a C1000, the S1000 can be controlled by:

- **The C1000 control panel**

  Connect the S1000 directly or indirectly to the C1000 through a USB cable, then program and run protocols using the C1000 user interface.

- **The 1000 series Manager software**

  Connect the C1000 to a computer via the C1000's USB B port, then control the C1000 and any connected S1000s with the 1000 series software.

The following three sections describe how to connect and operate an S1000 using a C1000 cycler:

- **Connect directly to a C1000 via the C1000 USB A port (below)**
- **Connect indirectly to a C1000 via the S1000 USB A port (page 106)**
- **Operating the S1000 while under the control of the C1000 (page 107)**

For detailed instructions about running the S1000 when it is connected to a C1000 see the instructions in the C1000 manual, or the 1000 series software online help or manual.

Connecting Directly to a C1000 via the C1000 USB A port

To connect the S1000 directly to a C1000 follow these instructions:

1. **Plug a USB cable into the S1000**

   Plug a high quality, shielded USB cable into the USB B port on the back of the S1000 (page 8). For USB cable part numbers, see page 118.

2. **Plug the USB cable into the C1000**

   Plug the other side of the USB cable into a USB A port on the back of the C1000. The C1000 will detect the S1000.

3. **Connect additional S1000s directly to the C1000**

   Repeat steps 1 and 2, to connect up to three S1000s directly to the same C1000.

4. **Open the `MAIN` screen or instrument tree on the C1000**

   Open the `MAIN` screen or instrument tree on the C1000, or in the 1000 series software if the C1000 is connected to a computer.

5. **Select the S1000**

   Select the S1000 by serial number or name. If the S1000 has a name, then the name will be displayed instead of the serial number.

Connecting Indirectly to a C1000 Thermal Cycler via the S1000 USB A Port

The S1000 can function under the control of the C1000 thermal cycler even when it is connected indirectly. To accomplish this follow these instructions:

1. **Plug a shielded USB cable into the S1000**

   Plug a high quality, shielded USB cable into the USB B port on the back of the S1000 (page 8). A shielded USB cable can be ordered directly from Bio-Rad.

2. **Plug the USB cable into the C1000**

   Plug the other side of the USB cable into a USB A port on the back of the C1000. The C1000 will detect the first S1000 thermal cycler.

3. **Connect another S1000 thermal cycler indirectly to the previous S1000**

   Plug a high quality, shielded USB cable into the USB B port on the back of another S1000 thermal cycler. Plug the other end of the cable into a USB A port on the previous S1000 thermal cycler (the S1000 connected directly to the C1000).

4. **Connect an additional S1000 indirectly to the C1000**

   By repeating steps 1-3, you may connect up to three S1000s indirectly to the same C1000.

5. **Open the instrument tree**

   Open the instrument tree on the C1000 or in the 1000 series software.

6. **Select the S1000**

   Select the S1000 by serial number or name. If the S1000 has a name, then the name will display instead of the serial number.

Operating the S1000 while under C1000 control

When the S1000 is under the control of the C1000, it is in "Semi-lock down mode".

In Semi-lock down mode, the S1000 will not respond when control panel keys are pressed. However, the following keys function on the control panel:

- SCREEN **key: Press this key to access the Running Screen, Graphical Screen, and Time Remaining Screen.**
- PAUSE **key: Press this key to temporarily stop a protocol that is currently running on the S1000**
- ENTER **key: Press this key to begin a run that has been remotely sent from the C1000**
- ENTER **key: Press this key to skip a step**
- CANCEL **key: Press this key to cancel a protocol that is currently running on the S1000**

Automated Control for Robotic Systems

The 1000-series is capable of responding to robotic control commands in a high-throughput workflow. To connect the C1000 to a robotic system, use a USB port.

Once connected, robotic commands automate basic operations of the C1000. For information about these commands, download the *Automation Guide* **from the Gene Expression Gateway (www.bio-rad.com/genomics). This guide includes instructions and commands for running C1000s under the control of a robotic system.**

Appendix A: Preinstalled Protocols

The C1000 comes with preinstalled protocols that provide a template for running new PCR experiments. Use these protocols as templates. The following sections describe the preinstalled protocols, and include tips about when to use them:

- Standard 2 and 3-step protocols (below).
- Touchdown PCR protocol to avoid non-specific primer binding (page 110).
- iTaq protocols optimized to run with iTaq™ hot-start polymerase (page 110).
- iProof protocols optimized to run with iProof™ high-fidelity polymerase (page 111).
- Reverse transcription protocols to amplify DNA from an RNA template (page 112)
- Nested primer protocols to amplify a specific DNA sequence from a large, complex DNA template (page 112).

Standard Protocols

Two standard protocols that run two-step and three-step PCR with a standard DNA polymerase. Choose these protocols to begin running PCR with new primers and DNA template, or copy them to begin writing a new protocol. Table 14 lists the parameters in each protocol.

Table 14. Standard Protocol*

	STD2		STD3	
	To Run a Standard Two-Step Protocol		To Run a 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

Table 14. Standard Protocol*

6	4	Infinite hold ∞	72	1:00
	END		4	Infinite hold ∞
7			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 15 lists the parameters in each protocol

Table 15. Touchdown Protocol*

	TCHDOWN	
	To run a protocol with an increment temperature step that automatically finds the most stringent conditions for primer binding	
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	4	Infinite hold ∞
12	END	

iTaq Polymerase Protocols

iTaq DNA polymerase is an antibody-mediated hot-start polymerase that is suitable for both PCR and real-time. The iTaq protocols run PCR using the optimal parameters

for this polymerase and associated buffers. iTaq polymerase is designed to be activated during the first step at 98°C and to amplify small to medium-size templates.

Table 16. iTaq Protocol*

	iTAQ-FST	
	To Run Fast PCR	
Step	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
1	98	0:30
2	92*	0:01
3	70	0:10
4	GOTO 2	29x
5	72	0:30
6	END	

iProof Polymerase Protocols

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. These iProof protocols include optimal parameters for this 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 17 lists the parameters in each protocol.

Table 17. iProof Protocol*

	IPRF1KB		IPRF8KB		IPRF15KB	
	To amplify 1 kb or smaller targets fast		To amplify 8 kb or smaller targets		To amplify 15 kb or smaller targets	
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
	4	Infinite hold ∞	4	Infinite hold ∞	4	Infinite hold ∞

Table 17. iProof Protocol*

	END		END		END	

IScript Polymerase Protocol

Table 18. IScript Protocol

Step	Target Temperature (°C) or GOTO Step	Time (min:sec) or Repeats
1	25	5:00
2	42	30:00
3	85	5:00
4	4	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 19 lists the parameters in each protocol.

Table 19. Nested Primer Protocol*

	NESTPR2		NESTPR3	
	To Run Two-step Protocol Using Nested Primers		To Run a 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	4	Infinite hold ∞	72	7:00
7	95*	0:30	4	Infinite hold ∞
8	65	0:30	95	0:30
9	GOTO 7	39x	55	0:30
10	72	7:00	72	0:30

Table 19. Nested Primer Protocol*

11	**4**	**Infinite hold** ∞	**GOTO 8**	**39x**
12	**END**		**72**	**7:00**
13			**4**	**Infinite hold** ∞
14			**END**	

Appendix B: Operating Specifications

The C1000 thermal cycler is designed for repeatable, consistent results. It heats and cools very fast during operation. Users should follow the operating specifications listed in this section to achieve reliable results:

- Instrument operating specifications and recommendations
- Gradient specification

Instrument Operating Specifications

This thermal cycler is designed to operate safely in a standard research laboratory for research applications.

NOTE: This instrument is for research use only.

For safe and reliable results, the operation of this instrument must follow the specifications listed in this manual. To ensure safe operation, follow these recommendations:

- Place the thermal cycler base on a flat surface
- Do not block fans during operation; keep the area around the instrument clear of objects or debris that could block air flow
- Read this manual for best usage practices
- Never remove the instrument's outer casing
- Do not operate in extreme humidity where condensation can impede proper operation
- Do not operate at extreme altitude
- Use only the provided power cord

Table 20. Operating specifications

Requirement	Specification
Thermal Block Uniformity	±0.4°C well-to-well uniformity at 90°C within 10 seconds
Thermal Accuracy	±0.2°C of programmed target temperature
Maximum Temperature	100°C

Table 20. Operating specifications

Requirement	Specification
Minimum Temperature	0°C
Max Ramp Rate	5°C/second
Heated Lid Range	0-110°C
Dimensions	18” x 11” x 12.5”
Weight	25.5 lbs
Line Voltage	100-240 VAC rms (no adjustment needed among voltages within these ranges)
Frequency	50-60 Hz single phase
Input Power	400 W max
Fuses	Two 6.3 A, 250 V, 5 x 20 mm
Display	12 x 9 cm, high resolution, full color
Ports	5 USB-A, 1 USB-B

Gradient Specifications

This thermal cycler can generate a temperature gradient across a 96-well or dual 48-well block. To view the estimated temperature in each well, use the GRADCALC **tool.**

Table 21. Gradient specifications

Requirement	Specification
Gradient Direction	Back (lower temperature) to front (upper temperature) of block
Maximum Gradient	30°C to 100°C
Gradient Temperature Differential	1-24°C
Gradient Uniformity	±0.4°C well to well uniformity within 10 seconds of step initiation
Gradient Accuracy	±0.2°C of programmed target temperature
Gradient Calculator Accuracy	±0.4°C of the actual well temperature

Appendix C: Catalog Numbers

This appendix lists useful catalog numbers and descriptions for the following components and accessories in the 1000-series:

- 1000-series components and descriptions (below)
- Accessories for the 1000-series with descriptions

Components of the 1000-series

Table 20 lists the components of the 1000-series instruments and software with the descriptions and catalog numbers:

Table 22: Catalog numbers for the 1000-series instruments and software.

Product	Description	Catalog number
Thermal cycler bases and reaction modules		
C1000 base	Thermal cycler base for PCR and real time PCR	184-1000
S1000 base	Thermal cycler base for PCR	184-2000
96 reaction module	96-well block for PCR with adjustable, heated lid	184-0096
Dual 48 reaction module	Two 48-well blocks for PCR with adjustable, heated lid	184-0048
384 reaction module	384-well block for conventional PCR with adjustable, heated lid	184-0384
C1000 96-well Fast System	C1000 chassis and 96 Fast reaction module system	185-1096
C1000 48/48 Fast System	C1000 chassis and 48/48 Dual Fast reaction module system	185-1048
C1000 384-well System	C1000 chassis and 384-well reaction module system	185-1384

Table 22: Catalog numbers for the 1000-series instruments and software.

Product	Description	Catalog number
S1000 96- well Fast System	S1000 chassis and 96 Fast reaction module system	185-2096
S1000 48/48 Fast System	S1000 chassis and 48/48 Dual Fast reaction module system	185-2048
S1000 384-well System	S1000 chassis and 384-well reaction module system	185-2384
Real time detection modules		
CFX96 Real Time Detection module	Real time detection module with 96-well block	184-5096
Software		
C1000 Manager software	Software package to run PCR with one or more thermal cyclers in a single window	184-4000
CFX Manager software	Software package to run real-time PCR and PCR with one or more thermal cyclers in a single window	184-5000
CFX Manager Security Edition software	Software package to run real-time PCR and PCR with one or more thermal cyclers in a single window. Includes security features for CFR part 11 compliance	184-5001

Accessories for the 1000-series

Table 21 lists accessory parts for the 1000-series instruments. It includes descriptions and catalog numbers:

Table 23: Catalog numbers of accessories to the 1000-series.

Product	Description	Catalog number
USB cable	Shielded USB cable to connect the S1000 directly to C1000.	184-8000
Tube Tray	Provides structural support for one or a few tubes	184-7000

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Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400*
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