

RoboCycler® Temperature Cyclers and Accessories

INSTRUCTION MANUAL

IN #70183-00

Revision #032002

Cautions *Keep the temperature cycler's safety cover closed for protection against burns from the hot blocks.*

Load and unload tubes carefully to avoid injury.

AUTHORIZED THERMAL CYCLER

This instrument, Serial No. (see label below) is an Authorized Thermal Cycler.

[label with serial number]

Its purchase price includes the up-front fee component of a license under United States Patent Nos. 4,683,195, 4,683,202 and 4,965,188, owned by Roche Molecular Systems, Inc., and under corresponding claims in patents outside the United States, owned by F. Hoffmann-La Roche Ltd, covering the Polymerase Chain Reaction ("PCR") process, to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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A service agreement plan can be purchased to extend the standard one-year warranty for the RoboCycler® temperature cycler and Hot Top Assembly. For one year, the RoboCycler temperature cycler and Hot Top Assembly will be protected through top-quality factory service, priority repair, and a system loaner program. The service agreement is available through Stratagene's National Service Center in Cedar Creek, Texas, USA. For more information, call the Stratagene Technical Services Department at 1-800-894-1304, and please mention the model of the RoboCycler temperature cycler when calling.

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	0800 023 0446	0800 023 0447	0800 023 0448
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	0800 830 250	0800 825 225	
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
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	0800 585 370	0800 783 0889	

All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

RoboCycler® Temperature Cyclers and Accessories

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RoboCycler® Temperature Cyclers and Accessories

MATERIALS PROVIDED

Material provided ^a	Quantity
RoboCycler® temperature cycler	1
Power cable	1
Safety cover	1
Hardware	
8-32 × 0.62-inch Phillips® screws	4
No. 8 flat washers and star washers	4 each
0.25 × 0.88-inch cap-head screw	1
3/16-inch hex wrench	1
Phillips®-head screwdriver	1

^a For information about materials provided with the Hot Top Assembly, please see Appendix I.

RoboCycler Catalog Numbers

Item	Catalog #
RoboCycler® 40 temperature cycler	400830 (100/120 V), 400832 (230 V)
RoboCycler® Gradient 40 temperature cycler	400860 (100/120 V), 400862 (230 V)
RoboCycler® 96 temperature cycler	400870 (100/120 V), 400872 (230 V)
RoboCycler® Gradient 96 temperature cycler	400880 (120 V), 400881 (100 V), 400882 (230 V)
RoboCycler® Gradient 40 temperature cycler with the Hot Top Assembly	400864 (100/120 V), 400866 (230 V)
RoboCycler® Gradient 96 temperature cycler with the Hot Top Assembly	400884 (120 V), 400885 (100 V), 400886 (230 V)

ADDITIONAL MATERIALS REQUIRED

ART® tips

Mineral oil or paraffin oil

Note *Stratagene offers a complete line of disposables for use with the RoboCycler temperature cyclers. To maximize temperature cycling performance, Stratagene's Thin-Wall Tubes are precision manufactured to meet the tightest industry specifications—those required for robotic systems. Stratagene strongly recommends using these tubes because the tubes are optimized and tested to assure ideal contact with the temperature blocks. See www.stratagene.com for more information on disposables.*

For 96-Well RoboCycler Temperature Cyclers

Thin-wall tubes, (200- μ l)

Thin-wall tube strips with caps, (200- μ l), 8- or 12-tube

96-well polypropylene plates with caps

100 precut pieces of plate-sealing foil (for use with polypropylene plates and the FastTherm™ Heat Sealer)

96-well polycarbonate plates (not for use with the Hot Top Assembly)

100 precut pieces of sealing tape for use with polycarbonate plates

96-tube transfer racks and 96-tube benchtop working racks

For 40-Well RoboCycler Temperature Cyclers

Thin-wall tubes, (600 μ l)

40-tube transfer racks and 40-tube benchtop working racks

INTRODUCTION

The RoboCycler® temperature cycler is a microprocessor-controlled, four-block laboratory instrument utilizing a robotic arm to quickly move samples from one temperature block to another, based on a user-defined program. The system has four anodized aluminum blocks, each containing 40 or 96 precision-cut wells. The system is capable of holding microfuge tubes or polypropylene plates. The unique four-block design decreases cycling time by up to thirty percent and achieves well-to-well temperature uniformity of $\pm 0.1^{\circ}\text{C}$ across the three hot blocks.^{1,2,3}

The system is programmed via a keypad with a built-in beeper that alerts the user when a key selection has been recognized. Data is displayed in a liquid crystal display (LCD) (see Figure 2). Temperature and dwell time for each block can be programmed through four sequentially linked windows.

- Blocks 1–3 can be programmed for temperatures between 25°C and 99°C .
- Block 4, the cold block, can be programmed for a temperature range of 6 – 25°C .
- Dwell times range from 1 second to 99 minutes, 59 seconds.
- Each window can be programmed to cycle up to 99 times.
- Each group of four windows (i.e., one program) can be stored permanently in one of 99 program storage areas. Program information may be easily reviewed or retrieved through a user-friendly display interface.
- Up to ten programs can be linked together to run sequentially.

The Hot Top Assembly for the RoboCycler temperature cycler eliminates the need to add an oil overlay to thin-wall reaction tubes by maintaining a temperature that reduces the condensation formed in the tubes during temperature cycling (See Appendix I).

If the temperature cycler is a gradient model,* Block 2, the special brass gradient block, can be programmed for a temperature gradient within the range of 35 – 99°C (45 – 99°C for a RoboCycler 96 temperature cycler with the Hot Top Assembly) with a well-to-well temperature uniformity of $\pm 0.25^{\circ}\text{C}$.² A linear temperature gradient is created by heating one end of the brass block and cooling the other end. The maximum gradient range across Block 2 is dependent on the model of RoboCycler temperature cycler being used (see Appendix II).

* U. S. Patent Nos. 5,525,300 and 5,779,981.

The RoboCycler temperature cycler can be used to perform incubations at one or more temperatures with precise time and temperature control. It is convenient for any reaction where a hot water bath or hot block incubator is currently used. When set at 14°C, the cold block (Block 4) is excellent for ligations. Blocks 1, 2, and 3 can be used as hot dry baths.

To use the RoboCycler temperature cycler as a hot or cold dry bath for 1.5-ml or 0.5-ml tubes, place Stratagene's Thermal Transfer Block on top of one of the hot blocks or on the cold block (see Appendix III). The Thermal Transfer Block includes 24 wells for 1.5-ml tubes and 14 wells for 0.5 ml tubes and has a temperature well for a glass bulb thermometer. An insulation jacket allows the block to be easily removed and transferred to the lab bench.

The RoboCycler temperature cycler can be programmed and operated from an external computer device via RS232C serial communication. A card is inserted into the temperature cycler to provide a serial interface with an external device. All RoboCycler temperature cycler programming, functions, and operation can be controlled by this interface. Please see www.stratagene.com for more information.

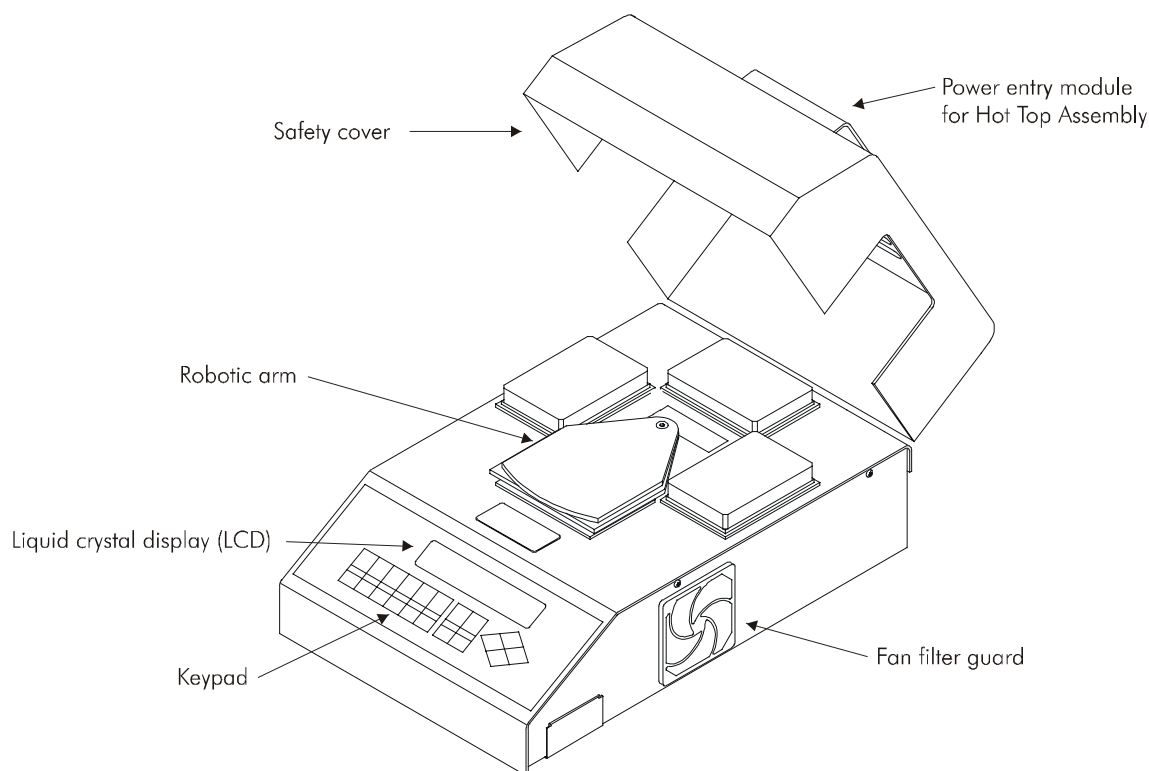


FIGURE 1 RoboCycler temperature cycler.

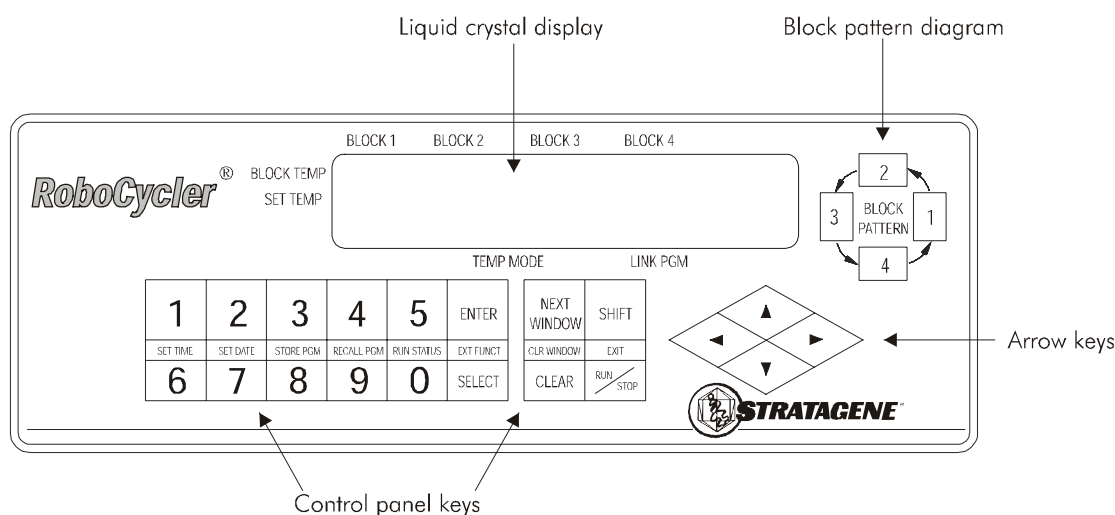


FIGURE 2 Control panel of the RoboCycler temperature cycler.

AUTOMATIC FEATURES OF THE ROBOCYCLER TEMPERATURE CYCLER

Automatic Position Sensing

The robotic arm automatically stops if it encounters resistance. The position-sensing robotic arm will reorient itself and move to the appropriate block if the arm is accidentally bumped mid-cycle.

Temperature Change Compensation

The RoboCycler temperature cycler automatically compensates for temperature changes which can occur when most or all of the wells are used. For example, when the arm moves tubes from the cold block (Block 4) to the first hot block (Block 1) on the first cycle of the program, the temperature of Block 1 may drop by as much as 4°C. The system compensates by pausing the countdown and waiting momentarily at Block 1 until this block reheats to within a degree of the set point.

When the tubes move from Block 1 at, for example, 95°C, to Block 2 at 54°C, the temperature of Block 2 may rise as much as 3°C. On the first cycle, the system will measure the temperature increase and adjust the set point accordingly for future cycles for that block, in this case decreasing the set point.

Sample Cooling

The thermoelectric Peltier cooling unit of Block 4 can be activated for pre- and post-run sample cooling. When a preheat sequence is started, the unit will begin cooling to the programmed temperature in the active window. If a time has been programmed for the cold block, the unit will continue cooling while running. Otherwise, the unit will shut off and begin cooling again with 30 minutes left in a program. When the program finishes, the block will be at or near the programmed temperature of Block 4 in Window 4. At the end of every program, the robotic arm will automatically place the tubes in the cold block, which will remain at the set temperature until turned off.

Built-In Safeguards

The RoboCycler temperature cycler is designed to conform to international safety specifications. It is equipped with an over-temperature cut-out device in each of the three hot blocks. If a fault occurs which might cause a heater to stay on, power is cut to the heater when it reaches a temperature of approximately 110°C. The thermal cut-out device will then automatically reset itself at approximately 90°C.

INSTALLING THE SAFETY COVER

Attach and tighten the safety cover using the Phillips screws and washers supplied (see Figure 3).

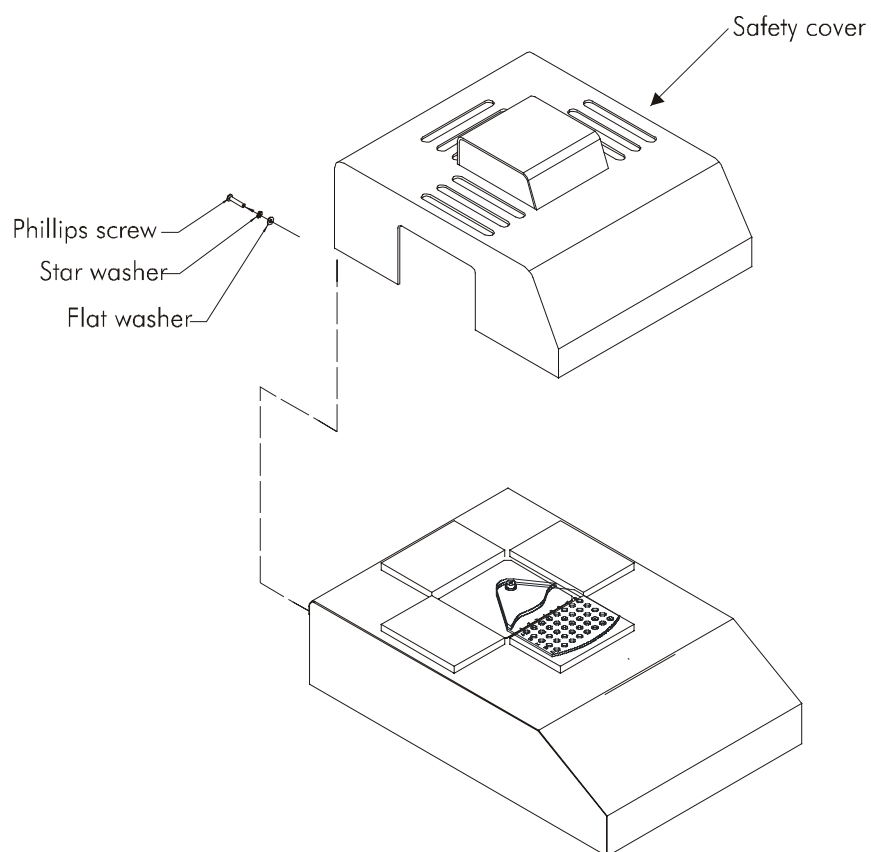


FIGURE 3 Assembly order of the safety cover on the RoboCycler temperature cycler.

USING THE ROBOCYCLER SOFTWARE

Windows

One window is displayed at a time on the LCD. A sample window is shown below.

	BLOCK 1	BLOCK 2	BLOCK 3	BLOCK 4	
BLOCK TEMP	21°C	21°C	21°C	21°C	WINDOW 4
SET TEMP	95°C	55°C	25°C	6°C	PROG# 1
	00:00	00:00	10:00	00:00	CYCLES 1
	REMAIN TIME 00:00		TRM=1	LPGM=OFF	CYCLE 1
	TEMP MODE		LINK PGM		

FIGURE 4 Sample program window of the RoboCycler temperature cycler

Each window consists of four lines of information:

- Line 1 indicates the actual temperature of each block in degrees Celsius as measured by the microprocessing system, and the window number.
- Line 2 indicates the user-programmed temperature of each block and the program number.
- Line 3 indicates user-programmed segment times (the length of time the robotic arm spends at each block) in minutes and seconds. This line also shows the user-programmed number of cycles for the window.
- Line 4 indicates run mode information. RUNTIME is the total hours and minutes (rounded to the nearest minute) remaining in a program (the total of programmed time and paddle movement in all four windows). If program linking is enabled, RUNTIME includes all linked programs. The number above “TEMP MODE” indicates which temperature running mode is active (see *Selecting a Temperature Run Mode*). If the temperature cycler is gradient-equipped, the gradient mode (ON or OFF) is shown (see Appendix II for more information). Either ON or OFF is displayed above “LINK PGM,” showing if programs linking is active (see *Linked Programs*). CYCLE indicates the cycle number running in the current window.

Programs

A RoboCycler temperature cycler program consists of four individual windows, each consisting of block temperatures, time periods, and repetitive cycles that define a particular experiment protocol. The execution of a program progresses from Window 1 through Window 4. The program ends at the conclusion of Window 4 and the RoboCycler temperature cycler places the paddle in Block 4 and maintains the programmed block temperature.

The Block 4 cooling mechanism is enabled only when time has been programmed into Block 4 for the currently active running window or when there is less than 30 minutes remaining in the programmed protocol. At the end of every program, Block 4 is maintained at the temperature setting for Block 4 in Window 4.

Multiple time and temperature profiles can be created by linking programs together. Program linking provides for up to 10 stored programs (40 windows) to be automatically run in sequence (See *Linked Programs*).

Examples

The following templates are provided as a visual aid in understanding the RoboCycler temperature cycler program in terms of windows, blocks, and repetitive cycles.

Program Window Template

Individual windows are programmed to create a specific repetitive temperature and time sequence. The blocks heat to the programmed temperature. The paddle then moves to successive blocks, remains in each block for the specified time, and repeats the sequence for the programmed number of cycles.

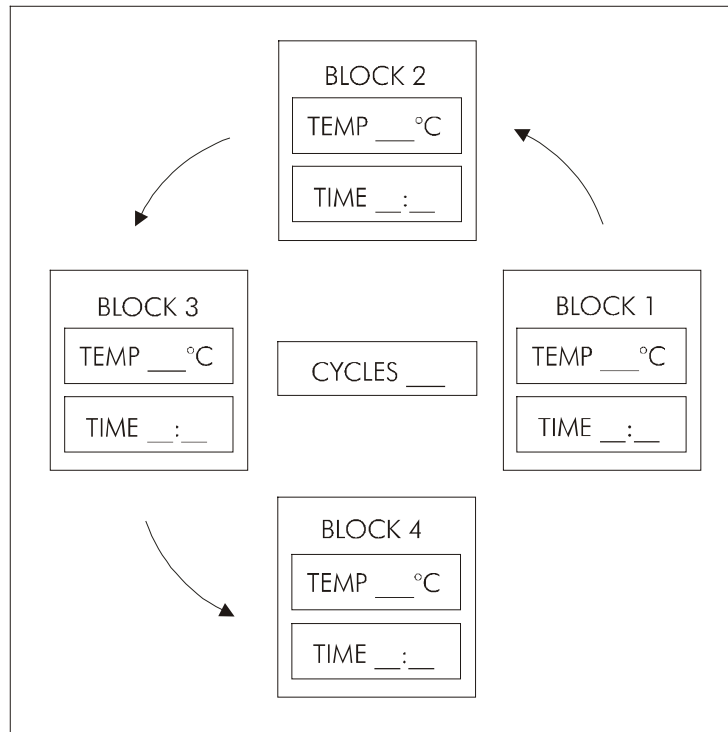


FIGURE 5 Template of a window of the RoboCycler temperature cycler.

RoboCycler Temperature Cycler Program Template

RoboCycler temperature cycler programs consist of four individually programmed windows that define a specific temperature/time experiment profile. The programming of each window is executed sequentially. If there are no block time or cycles programmed in a particular window, that window is skipped.

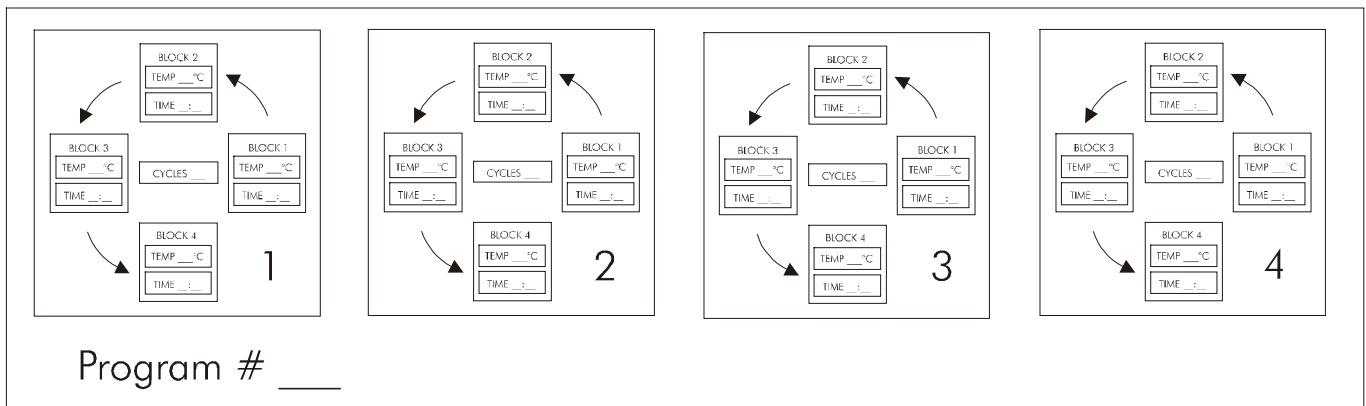


FIGURE 6 Template of a program of the RoboCycler temperature cycler.

RoboCycler Temperature Cycler Linked Programs Template

Up to 10 programs (40 windows) can be linked together to form more extended experiment protocols. Linked programs are recalled automatically from RoboCycler temperature cycler memory and must be previously stored.

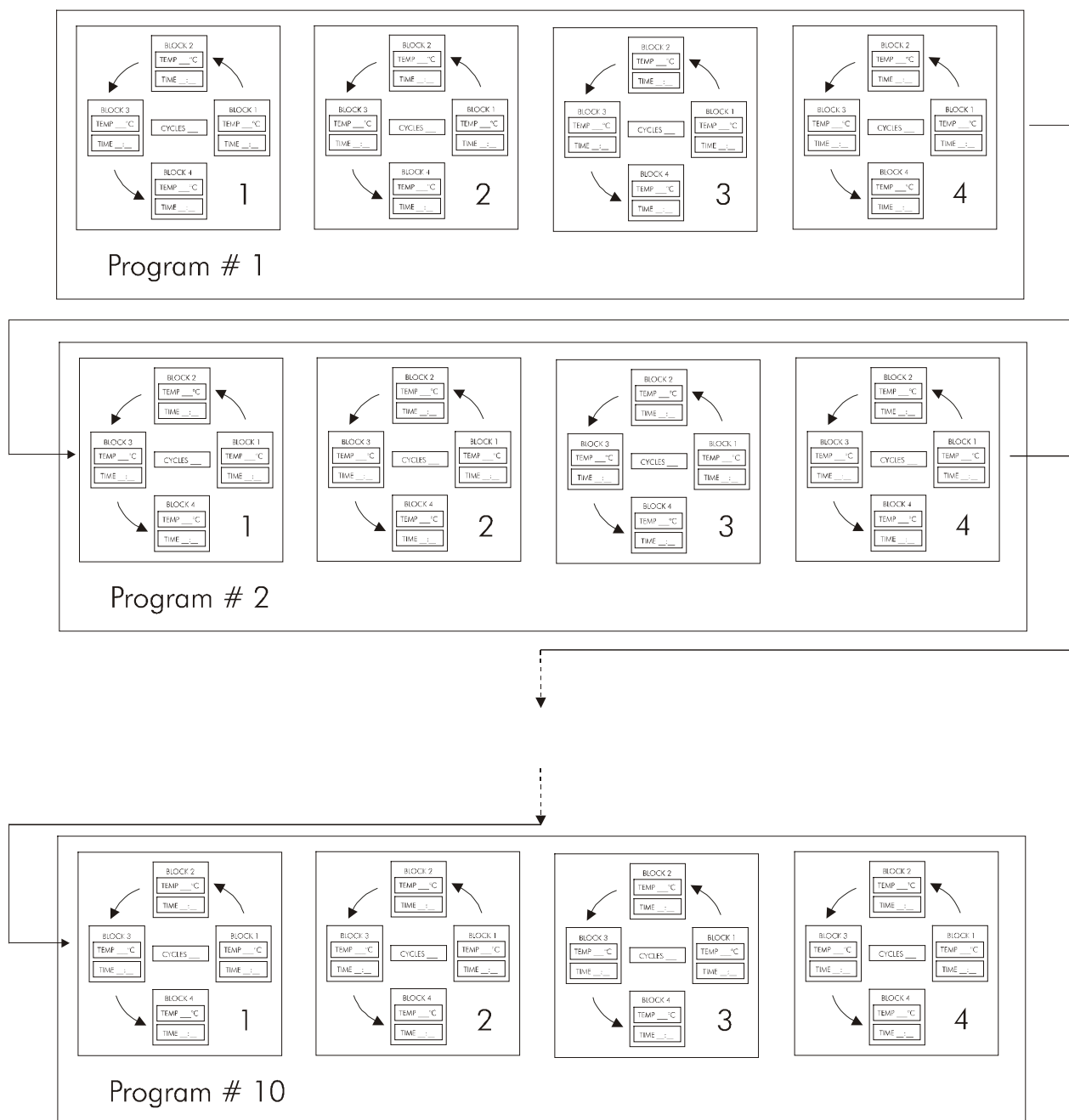


FIGURE 7 Template of a linked-program series of the RoboCycler temperature cycler.

OPERATING THE ROBOCYCLER TEMPERATURE CYCLER

See the *Quick-Reference Command List* at the end of this manual for a detachable reference sheet. Also see the following sections:

- ♦ General Programming Information
- ♦ RoboCycler Programming Worksheet

Cursor Movement

The RoboCycler temperature cycler is programmed through a keypad on the front face of the instrument. The cursor moves in the following fashion:

- ♦ Automatically, during programming, space-by-space within fields, and field to field.
- ♦ By using the arrow (cursor) keys to move the cursor space by space within fields, and field to field, in any direction.
- ♦ By pressing ENTER to advance the cursor from field to field.
- ♦ By pressing the NEXT WINDOW key to move from window to window. After Window 4, the display returns to Window 1.

Modes

Four different software modes allow the user to view and manipulate programming information in the RoboCycler system.

- ♦ EDIT mode allows the user to view, edit, and store any parameters or programs. All functions are available in the EDIT mode. The system is in EDIT mode when initially turned on.
- ♦ RUN mode is activated when a program is started. The only commands active in RUN mode are RUN/STOP and SHIFT + 5 (Toggle Hot Top Assembly Off and On).
- ♦ PAUSE mode is activated when a running program is paused by pressing the RUN/STOP key. In PAUSE mode the only options available are exiting the program or going into the EDIT/REVIEW mode.
- ♦ EDIT/REVIEW mode is entered from a paused program. In this mode all windows of a paused program may be reviewed but only those windows which have not yet been active may be edited.

Smart Programming

For maximum efficiency, Stratagene recommends careful selection of blocks and temperatures so the change in block temperature from window to window is minimized. The RoboCycler temperature cycler is faster than conventional temperature cyclers because it does not have block ramping times. As the RoboCycler temperature cycler changes windows, the individual blocks are brought to the programmed temperature of the new window.

However, temperature ramping does occur within the tubes due to the thermal characteristics of the tubes and fluid. On average, the tubes and fluid change temperature at 1°C per second. The user may need to account for these factors and review the time needed for different steps. The system begins counting down once the tubes are placed into a block.

For example, if Window 1 only requires one 37°C block and Window 2 uses three blocks of 60, 68, and 95°C, arrange the program so the block that was the 37°C block only has to heat to 60°C. Program Window 1 to heat the unused blocks to their Window 2 temperatures of 68°C and 95°C.

When the program switches to Window 2, the 37°C block will begin heating to 60°C and the other blocks will be ready at their Window 2-programmed temperatures. Stratagene recommends running Block 2 as the annealing block (or the block with the lowest temperature). Block 2 is fan-cooled and will maintain the cooler temperatures for the annealing step even when a number of samples heated to 95°C are moved from Block 1 to Block 2.

General Programming Information

Turning a Window Off

While programming, the quickest way to cause a displayed window to not run (disable that window) is to press the *SHIFT* key, then press the *CLR WINDOW* (Clear Window) key. This will clear all data in the window (see *Clear Window* in the *Shift Functions* section). All temperatures will default to 25°C for the hot blocks and to 6°C for the cold block. All times will default to 0:00. The cycle number will default to 0.

A window may also be turned off by simply programming zeros into the cycle or segment times. If zero times are programmed for all blocks or zero cycles are programmed, the window is skipped.

Reviewing the Program

- Always check to see if the program being used has other windows programmed. To do this, scan all four windows using the *NEXT WINDOW* key. If other windows contain unwanted programming information, they can be cleared by pressing *SHIFT*, then pressing *CLR WINDOW* (Clear Window), or by entering zero cycle times in each of the unused windows.
- Before pressing *RUN/STOP*, be sure Window 1 is displayed so cycling will begin from Window 1. Pressing *RUN/STOP* will start execution at the currently displayed window, proceeding sequentially to Window 4.

Power Interruption

If a power interruption occurs during a running program, the system stores the status of the program at the time of the power interruption. When power returns, the paddle is rotated to the cold block, which is maintained at 6°C, and the following message is displayed:

NOTICE:	A Power Interrupt Occurred	
	Holding in Cold Block at 6°C	
SELECT	[RUN STATUS]	to display status
PRESS	[ENTER]	to continue

Select *RUN STATUS* to view the status of the program at the time of the power interruption. If the status is not immediately viewed, the power interrupt status is erased (see *RUN STATUS* in *Shift Functions*). Press *ENTER* to continue without viewing the status of the interrupted program. Window 1 of the interrupted program will be displayed but the program will not resume running from the time of power interruption; the program must be restarted.

Getting Started

The blocks of the RoboCycler temperature cycler are numbered 1 through 4 in a counterclockwise direction. Block 1 is on the right when facing the machine. The robotic arm always begins and ends a program at the cold block, Block 4. (See the upper-right-hand corner of Figure 2 for the block pattern diagram.)

1. Place the unit on a flat, stable surface, making sure air vents on the sides and rear are not obstructed. Plug the power cord into the rear of the temperature cycler and the other end into a grounded power outlet.

2. Turn the power switch located in the right rear of the machine ON. The LCD will identify the unit as *ROBOCYCLER* and display the current software level.

Every time the RoboCycler temperature cycler is turned on, the robotic arm runs a quick positional test called the auto-zero cycle, one quick rotation to ensure that the arm starts at the home position, Block 4 (the cold block). At the conclusion of the auto-zero cycle, the system automatically enters EDIT mode at Window 1.

3. A flashing cursor is displayed at the beginning of line 2, Block 1. The system is now in EDIT mode and is ready for programming.

Programming

Using the RoboCycler temperature cycler consists of three steps: creating a program, selecting a temperature run mode, and running the program.

Creating a Program

When the temperature cycler is first turned on, it automatically enters EDIT mode. The cursor is already at line 2 of Block 1 in Window 1, allowing the user to program the temperature. As temperatures are entered, the cursor advances from Block 1 temperature to Block 2, 3, and 4 temperatures. The cursor then moves down to line 3, Block 1 segment time, and advances to Blocks 2, 3, and 4 segment times as times are entered. Finally, the cursor will move to the end of Row 3 and allow programming the number of cycles for this window.

Note *Temperatures are recorded in degrees Celsius. Segment time is recorded in minutes and seconds and run time is recorded in hours and minutes.*

After Window 1 has been programmed, press the *NEXT WINDOW* key and Window 2 will appear. The same programming sequence may be repeated for Windows 2, 3, and 4.

Hint *Data already programmed in the current window can be copied into the next window by pressing the *SHIFT* key followed by the *NEXT WINDOW* key. The window copy feature is disabled when moving from Window 4 back to Window 1.*

Selecting a Temperature Run Mode

The RoboCycler temperature cycler has three different temperature running modes.

Temperature Run Mode 1: BEGIN RUNNING AT SET TEMP

Mode 1 should be used when the reaction tubes are ready for cycling and loaded into the cycler. Mode 1 sets the RoboCycler temperature cycler to heat the blocks and automatically begin cycling when the hot blocks reach their set temperatures. Press *RUN/STOP* to preheat the blocks; the program will start automatically at the set temperatures.

Temperature Run Mode 2: BEGIN RUNNING AT TEMP WITH ENTER

Mode 2 should be used as a preheating mode. Press *RUN/STOP* to preheat the blocks while preparing the reaction tubes. The blocks will reach their set temperatures and maintain these temperatures. Load the tubes into the robotic arm when ready to begin cycling. When the tubes are loaded into the temperature cyclers and the blocks have reached their set temperatures, press *ENTER* to begin running the program.

Temperature Run Mode 3: BEGIN RUNNING PROGRAM IMMEDIATELY

In Mode 3, pressing *RUN/STOP* begins a program immediately. The hot blocks may not be at their set temperatures.

Enter the mode as follows:

1. Press *SHIFT*, then *EXT FUNCT*. This brings up the Extended Functions menu.
2. Select *TEMP RUN MODE* by pressing *1*.
3. Select the desired temperature run mode.

Note *The cold block does not have to be at its set temperature for a program to begin.*

About the Hot Top Assembly

The RoboCycler temperature cycler does not detect when the Hot Top Assembly is at the proper starting temperature. The Hot Top Assembly takes less time to reach temperature than the blocks of the temperature cycler. Therefore, it will have reached proper starting temperature by the time the hot blocks have reached cycling temperature. The Hot Top Assembly can, however, be preheated by pressing *SHIFT*, then *EXT FUNCT*, then *5* in EDIT mode or *SHIFT*, then *5* in any mode. This will turn the Hot Top Assembly on without starting the temperature cycler. For more information about the Hot Top Assembly, see Appendix I.

Running a Program

Warning *Special precautions should be taken when using ³⁵S nucleotides, which may break down into a highly volatile compound when subjected to repeated high-temperature cycles. Accordingly, Stratagene recommends using ³³P instead of ³⁵S. If ³⁵S nucleotides must be used, the following safety guidelines are recommended:*

- a. Use only thin-wall tubes.
- b. Use oil in the block wells for efficient heat transfer with the thick-wall tubes.
- c. Minimize the number of temperature variations and cycles in the thermal cycling program.

1. Open the acrylic safety cover.

Note *Before operating the RoboCycler temperature cycler, add one or two drops of oil (enough to leave only a thin film of oil) into the wells of one of the blocks to enable efficient heat transfer (Block 1 is usually used for this purpose).*

2. Load tubes into the paddle of the robotic arm. If desired, the robotic paddle may be raised for insertion of tubes by pressing *SHIFT* and then the Arrow Up key. Pressing *SHIFT* then the Arrow Down key will lower the paddle. Make sure the paddle is down prior to selecting *RUN/STOP*. If using a 96-well polycarbonate plate, be sure to firmly push the plate all the way into the paddle.

Note *For protection against burns, keep the safety cover and the robotic arm safety lid or Hot Top assembly cover closed at all times except when handling tubes.*

3. Close the safety cover.

Note *Before pressing RUN/STOP, make sure Window 1 is displayed to ensure that cycling begins from Window 1.*

4. Press the *RUN/STOP* key (and/or *ENTER*, if using the *BEGIN RUNNING AT TEMP WITH ENTER* preheating mode, Mode 2) to begin running the program. The system is now in RUN mode.

During RUN mode, the window will display the current window and cycle number being executed. Programming data cannot be entered during RUN mode. The segment time corresponding to the currently running block will decrement to 0:00 before the next block is executed. *RUNTIME* indicates the time, in hours and minutes, remaining in all windows of the total program, and will decrement to 0:00 when <30 seconds remain in a program.

Thirty minutes before the end of a program, the cold block begins cooling down to the set temperature of Block 4 of the window that is currently running. When the RoboCycler temperature cycler completes execution of all valid windows in a program, the tubes are automatically moved to rest in the cold block. At this time, the program will go to Window 4 (if not already there) and Block 4 will cool to its set temperature in Window 4 and maintain this temperature until the user presses *ENTER* to turn the cold block off. The tubes can then be removed.

Note *Stratagene recommends turning the cold block off at the end of an experiment.*

Editing a Running Program

In the RUN mode, the currently running window may only be viewed, not edited. No other windows can be viewed. If you wish to view information in another window or to edit the program, you must either pause or exit the program.

Pausing a Program

The RoboCycler temperature cycler can be paused at any point in a program to edit, add enzymes, microfuge reaction samples, or perform other tasks. If a window has not yet been run, it may be modified in the EDIT/REVIEW mode.

In the PAUSE mode, the paddle can also be raised or lowered to access the reaction tubes (See *Raise Paddle/Lower Paddle*).

1. To pause a running program, press the *RUN/STOP* key. The system will then be in PAUSE mode. Line 4, which displays run mode information, will show available options, as in the example below:

95°C	55°C	72°C	6°C	WINDOW	1
95°C	55°C	72°C	6°C	PROG#	1
0:58	1:30	2:00	0:00	CYCLES	30
1 – Review/Edit Program, Exit = SHIFT EXIT					

2. Press *1* to select EDIT/REVIEW mode, which allows editing or reviewing of the subsequent windows without exiting the running program. The LCD will display the following:

95°C	55°C	72°C	6°C	WINDOW	1
95°C	55°C	72°C	6°C	PROG#	1
0:58	1:30	2:00	0:00	CYCLES	30
RUNTIME	00:00	1	OFF	*ED/REV	

3. Press the *RUN/STOP* key to save the new parameters and resume running the program from the point at which it was paused. To quit the program without saving the parameters, press *SHIFT*, then press *EXIT*.

Note When the software is in EDIT/REVIEW mode, temperature control is still active. Time does not decrement while in the EDIT/REVIEW mode.

Exiting and Editing a Running Program

If a running program needs to be changed after the run has started, it can be paused, edited, and resumed (See *Pausing a Program*) or cancelled, edited, and restarted.

To edit a portion of the run which has already been executed the program must be exited. Push *RUN/STOP*, then *SHIFT*, then *EXIT* to exit the program. The program can then be edited and restarted at the beginning of the displayed window. If the program is stopped (not paused), block temperatures are not maintained.

SHIFT FUNCTIONS

To access the built-in special features of the RoboCycler temperature cycler, press the *SHIFT* key, then press the appropriately named key.

Hint *It is not necessary to hold the SHIFT key down while pressing the next key in SHIFT FUNCTIONS. When the SHIFT key is activated, the “SHIFT” prompt appears in the lower right-hand corner of the LCD.*

Refer to Figure 2 for an illustration of the keypad (for gradient temperature cyclers, see Figure 2 in Appendix II). The following is a description of the features accessed through *SHIFT* functions.

Shift Functions

Feature	Keystroke Sequence
Set Gradient	SHIFT + SET GRAD
Clear Gradient	SHIFT + CLR GRAD
Set Time	SHIFT + SET TIME
Set Date	SHIFT + SET DATE
Store Program	SHIFT + STORE PGM
Recall Program	SHIFT + RECALL PGM
Run Status	SHIFT + RUN STATUS
Extended Functions	SHIFT + EXT FUNCT
Clear Window	SHIFT + CLR WINDOW
Copy Window	SHIFT + NEXT WINDOW
Toggle Hot Top Power On/Off	SHIFT + 5
Raise Paddle/Lower Paddle	SHIFT + ^ / SHIFT + v
Exit	SHIFT + EXIT

Set Gradient

SET GRAD allows the user to program the system in gradient mode. See Appendix II for more information.

Clear Gradient

CLR GRAD Allows the user to reset (clear) the gradient temperature settings. See Appendix II for more information.

Set Time

The base time of the instrument can be set for 12- or 24-hour mode. Pressing *SHIFT* then *SET TIME* causes the *SET TIME* display to appear:

CURSOR KEYS & ENTER SET TIME AND MODE

CURRENT TIME: 10:35:04 AM

EXIT = *1 = AM 2 = PM

SHIFT EXIT *3 = 12hr 4 = 24hr

* Current selection.

The cursor automatically positions itself at the first digit of the hour. Enter the current time using the number keys (use a 0 before numbers less than 10, e.g., 09:01:05). The cursor will automatically advance from the hour to minutes and seconds as each number is entered. To select *AM/PM* or *12/24* hour modes, move the cursor to the appropriate number (1–4) and press *ENTER*. To change an entry, use the arrow keys to move the cursor into the desired position and re-enter the number. (An error message displays if an invalid time is entered.) When the time has been correctly set, press *SHIFT* followed by *EXIT* to save the new time setting and exit.

Set Date

Allows the user to set the base date of the instrument in MM/DD/YY or DD/MM/YY format. The *SET DATE* display appears as follows:

CURSOR KEYS & ENTER SET DATE AND MODE

CURRENT DATE: 05/12/99

EXIT = *1 = mm/dd/yy

SHIFT EXIT 2 = dd/mm/yy

* Current selection.

The cursor will be positioned on the first month or day digit, depending on the type of date display already selected. Enter the month or day using the number keys (use a 0 before numbers less than 10, e.g., 05/04/99). The cursor will automatically advance from month to day (or day to month) to year, as each number is entered. To select mm/dd/yy or dd/mm/yy mode, move the cursor to the appropriate number (1 or 2) and press *ENTER*. To change an entry, use the *ARROW* keys to move the cursor into the desired position and re-enter the number. (An error message displays if an invalid date is entered.) When the date has been correctly set, press *SHIFT* followed by *EXIT* to save the new time setting and exit.

Store Program

Up to 99 programs can be stored in permanent memory. The *STORE PGM* display appears as follows:

95°C	55°C	72°C	6°C	WINDOW	1
press	[PROG# + ENTER]	to	store	PROG#	1
press	[SHIFT + ENTER]	to	exit function	CYCLES	30
RUNTIME	00:00	1	OFF	CYCLE	0

Enter the two-digit number of the program to be stored, from 01 to 99, and press the *ENTER* key. If the program number is not in use, the program will be permanently stored in memory. If the selected program number is already being used, the following message will appear on line two:

95°C	55°C	72°C	6°C	WINDOW	1
pgm in use, ENTER to overwrite PROG#					
0:58	1:30	2:00	0:00	CYCLES	30
RUNTIME	00:00	1	OFF	CYCLE	0

Press the *ENTER* key to replace the existing program in memory or select another program number.

Recall Program

Use the *RECALL PGM* feature to retrieve a program from permanent memory. The *RECALL PGM* display appears as follows:

95°C	55°C	72°C	6°C	WINDOW	1
press [PROG# + ENTER] to recall PROG#					
0:58	1:30	2:00	0:00	CYCLES	30
RUNTIME	00:00	1	OFF	CYCLE	0

Enter the number of the program to be recalled, from 01 to 99, and press the *ENTER* key. The contents of that program will then appear on the display.

Run Status

Allows the user to examine information about the last two programs run. When in the *RUN STATUS* mode, the display appears as follows:

RUN STATUS: up/dn scroll, ENTER to exit
1 ...Current Run Status
2 ...Previous Run Status
3 ...Power Interrupt Status

Select *1* to review the status of the last program run or *2* to review the status of the next-to-last program run. Selection *3* is displayed only when a power interruption has occurred. Use the arrow keys to select a choice and then press the *ENTER* key.

If *1* or *2* is selected, the *RUN STATUS* menu will display as follows:

RUN STATUS: up/dn scroll, ENTER to exit
START 05/11 11:54am
FINISH 05/11 12:02pm

If there are more than three lines of *RUN STATUS* information, use the up and down arrow keys to scroll through the display. When finished viewing, press the *ENTER* key to exit. A brief explanation of the status message follows:

Run Status Items

Item	Explanation
START	Time and date the program was started.
FINISH	Time and date the program successfully finished running.
EXIT	Time and date the program was terminated by the user.
PWR LOSS	Time and date power was lost to the instrument during program execution. ^a
PWR ON	Time and date power was restored. Program will not resume automatically. ^a

^a Displayed only if a power interruption occurred.

If Power Interrupt Status (3) is selected, information about the power interruption will be displayed as shown below. Note that this entire display will not fit into the window. The up and down arrow keys must be used to scroll through the information.

RUN STATUS: Up/Dn scroll, ENTER To Exit	
POWER INTERRUPT STATUS	
PWR LOSS	12/29 11:14 am
PROGRAM NUMBER	2
WINDOW NUMBER	1
CYCLE NUMBER	1
BLOCK NUMBER	1
ELAPSED TIME	0:01
RUNTIME	0:04

Power Interruption Display Information

Item	Explanation
PWR LOSS	Date and time of power loss/interruption.
PROGRAM NUMBER	Number of program in use during power interruption.
WINDOW NUMBER	Number of window in use during power interruption
CYCLE NUMBER	Number of cycle in use during power interruption.
BLOCK NUMBER	Number of block in use during power interruption.
ELAPSED TIME	Elapsed time in the block when power loss occurred.
RUNTIME	Time remaining in program when power loss occurred.

Extended Functions

The Extended Functions menu provides access to the six extended functions.

Extended Functions

Feature	Keystroke Sequence
Temp Run Mode	SHIFT + EXT FUNCT + 1
Pos Calibrate	SHIFT + EXT FUNCT + 2
Link Programs	SHIFT + EXT FUNCT + 3
Beep On/Off	SHIFT + EXT FUNCT + 4
Hot Top On/Off	SHIFT + EXT FUNCT + 5
Paddle Type	SHIFT + EXT FUNCT + 6 [°]
Reset Serial Communications	SHIFT + EXT FUNCT + CLEAR
Exit	SHIFT + EXT FUNCT + 7

[°] For RoboCycler 96 temperature cyclers only

Selecting 7 exits the menu.

Set Temperature Run Mode

See *Selecting a Temperature Run Mode*.

Position Calibration

Calibrating the position of the robotic arm ensures that the reaction tubes are aligned with the blocks of the temperature cycler. Position calibration should be used only when the paddle or Hot Top Assembly has been removed and reinstalled. For more information about the Hot Top Assembly and its installation, see Appendix I.

1. From the main display screen, press *SHIFT* and then press *EXT FUNCT* to open the Extended Functions Menu.
2. Select *POS CALIBRATE (2)*.
3. Insert two tubes into the paddle while the paddle is positioned in Block 4 (i.e., the cold block).
4. Follow the directions indicated on the display. The robotic arm will lift, rotate, and return to rest at the cold block.
5. Press *ENTER* to complete the calibration routine. The system will return to the Extended Functions menu. The robotic arm is now calibrated.
6. Exit the Extended Functions menu.

Linked Programs

This feature, which recalls programs from memory, allows execution of up to ten sequential programs (up to 40 windows).

Note *Linking of programs is automatically disabled at the end of each run and must be enabled again via the LINK PROGRAMS option in the Extended Functions menu.*

To access the Link Programs menu, select *Link Programs (3)* from the Extended Functions menu.

ENTER LINKED PROGRAM SEQUENCE AND MODE

Exit = 1 Enable Program Linking

Shift Exit * 2 Disable Linking

* Link mode currently selected.

---- No program number assigned (if a program has been entered, the program number will appear at the sequence location).

Use the arrow keys and the *ENTER* key to position the cursor in the desired linked-program sequence position and enter the program number (01–99). Programs run in sequence from left to right. Cleared program positions (shown as ____) are ignored.

Note Pressing *CLR WINDOW* (Clear Window) clears a program number at the cursor position. Pressing *SHIFT* and *CLR WINDOW* clears the entire linked-program sequence and disables *LINKED PROGRAMS*.

To select the desired Linked Program mode (*ENABLED* or *DISABLED*), use the down arrow to position the cursor and press *ENTER*. Press *SHIFT*, then press *EXIT* to exit the Linked Programs menu.

Linked Programs: General Programming Information

When enabling program linking, the first program in the linked sequence becomes the active program. However, linked program execution will begin from the program and window displayed when the *RUN/STOP* key is pressed.

Each linked program has its own temperature run mode. If Temperature Run Mode 1 (Begin running at set temp) is set for the next linked program in a sequence, the paddle will remain in the last programmed block of the current program until the next program's set temperatures have been reached, at which time the next program will immediately execute. If Temperature Run Mode 2 (Begin running at temp with enter) is set for the next program, *ENTER* must be pressed to execute the next program when the block temperatures are reached. If Temperature Run Mode 3 is set for a program, that program will begin regardless of whether the blocks have reached their set temperatures.

Editing

To modify any window or program in a linked sequence other than those windows of the current program which have not yet been run, the user must exit to the *EDIT* mode, modify and save the program, reconfigure the linked programs, and restart the linked program sequence.

- ♦ In a linked-program series that is running, those windows of the current program that have not yet been executed may be edited by pausing the program, editing the windows, and resuming the program (See *Pausing a Program*).
- ♦ To modify a linked program that has not yet been run after starting a linked program sequence, exit from linked programs to the *EDIT* mode, modify and save the program, reconfigure the linked programs, and restart the linked program sequence (See *Exiting and Editing a Running Program*). The linked sequence can be restarted from wherever the user wishes.
- ♦ To modify previously run programs or previously-run windows of the current program, exit from linked programs to the *EDIT* mode, modify and save the program, reconfigure the linked programs, and restart the linked program sequence (See *Exiting and Editing a Running Program*). The linked sequence can be restarted from wherever the user wishes.

RUNTIME indicates the total programmed time for the entire linked program sequence. Block preheating time is not calculated in the *RUNTIME* indication. Consequently, actual run time will be greater than the indicated *RUNTIME*.

After completing the last program in the linked sequence, the paddle returns to the cold block (Block 4) and holds, as in normal operation.

Turn Beeper ON/OFF

The RoboCycler temperature cyclers have a keypad with a built-in beeper that alerts the user when a key selection has been recognized. This feature can be toggled on and off by pressing 4 in the Extended Functions menu.

Note *A beep will sound in the event of an error condition. Turning the beeper off will not disable the beep due to an error condition.*

Paddle Type

The *Paddle Type* function allows the temperature cycler to be used with either the Hot Top Assembly or the standard paddle. Selection 1, *Hot Top Paddle*, configures the software of the temperature cycler for the Hot Top Paddle (See Appendix I). Selection 2, *Standard Paddle*, configures the software of the temperature cycler for the standard paddle or for a Hot Top paddle that is to be used as a standard paddle. This function is only present in RoboCycler 96 temperature cyclers.

Note *The standard paddle is not provided if the RoboCycler temperature cycler was purchased with a Hot Top Assembly installed.*

Clear Window

Clear all programmed parameters in a window by pressing *SHIFT* then *CLR WINDOW*. All temperatures will be returned to default values of 25°C for the hot blocks and 6°C for the cold block.

Copy Window

Pressing *SHIFT* and then *NEXT WINDOW* copies programmed data to the *NEXT WINDOW*. To prevent inadvertent programming errors, the copy window capability is disabled when wrapping back to Window 1 from Window 4.

Raise Paddle/Lower Paddle

The robotic arm paddle can be raised to allow insertion or removal of tubes and then lowered to run the program. The paddle can be raised for insertion of tubes by pressing *SHIFT* and then the Arrow Up key. Pressing *SHIFT* then the Arrow Down key will lower the paddle. Raising and lowering of the paddle is allowed only during EDIT, EDIT/REVIEW, or PAUSE modes. Ensure the paddle is down before selecting *RUN/STOP*.

Toggle Hot Top Power On/Off

To turn the power to the Hot Top Assembly on or off at any time, press *SHIFT* then 5. This feature is useful for preheating the Hot Top Assembly and for disabling it. See Appendix I for more information.

APPLICATIONS

Polymerase Chain Reaction (PCR)

The following is a sample protocol for a PCR reaction to be run on a RoboCycler 96 temperature cycler. For more information on this protocol, please see Stratagene's *PfuTurbo™ DNA Polymerase Instruction Manual*. Optimal PCR conditions must be determined experimentally by the researcher.

Note *Please refer to Sample PCR Parameters and Sample PCR Program Entry sections immediately following this procedure for detailed instructions on how to program cycling parameters into the RoboCycler temperature cycler.*

1. Place 50–100 ng of genomic DNA (1–10 ng of plasmid DNA) in a sterile 200- μ l thin-wall microfuge tube.

2. Add the following components in the stated order:

5.0 μ l of 10 $\times$ Taq DNA polymerase buffer[†]
0.4 μ l of 25 mM dNTPs
1.0 μ l of primer #1 (100 ng/ μ l)
1.0 μ l of primer #2 (100 ng/ μ l)
0.5 μ l PfuTurbo DNA Polymerase (2.5 U/ μ l)
Sterile water to a final volume of 50 μ l

Notes *Labeling tubes on top, rather than the side, is recommended because the oil may remove ink markings made on the sides of tubes.*

Before operating the RoboCycler temperature cycler, Stratagene recommends adding one or two drops of oil (enough to leave only a thin film of oil) into the wells of one of the blocks to enable efficient heat transfer (usually Block 1 is used for this purpose).

[†] See Endnotes.

3. Load the tubes into the paddle of the robotic arm. Program the temperature cycler using the parameters in the Sample PCR Parameters table below. Push *ENTER* to begin the program.

Note *At the end of the thermal cycling procedure, the robotic arm of the RoboCycler temperature cycler will move to the prechilled cold block and maintain its set temperature until ENTER is pressed (turning off the cold block).*

4. When the thermal cycling procedure is complete, press *ENTER* to turn off the cold block. Remove the tubes from the machine.
5. Analyze a 10- μ l aliquot from the amplified sample via electrophoresis, using agarose, acrylamide, or VisiGel™ separation matrix and suitable molecular weight markers.

Sample PCR Program

The first sample below, *Sample PCR Parameters*, illustrates an example of thermal cycling parameters for PCR. The second sample, *Sample PCR Program Entry* shows how to program these parameters in to the RoboCycler temperature cycler. For information on gradient programs, see Appendix II.

Sample RoboCycler PCR Parameters^a

Window	Block 1	Block 2	Block 3	Cycles
1	95°C/ 1:00 min.	—	—	1
2	95°C/1:00 min.	56°C/ 1:00 min.	72°C/3:00 min.	30
3	—	—	72°C/7:00 min.	1
4	—	—	—	—

^a At the end of the program, the tubes will automatically hold in the cold block at 6°C until *Enter* is pressed.

Sample PCR Program Entry

21°C	21°C	21°C	21°C	WINDOW	1
95°C	56°C	72°C	6°C	PROG#	1
1:00	0:00	0:00	0:00	CYCLES	1
RUNTIME	3:26	1	OFF	CYCLE	0

21°C	21°C	21°C	21°C	WINDOW	2
95°C	56°C	72°C	6°C	PROG#	1
1:00	1:00	3:00	0:00	CYCLES	30
RUNTIME	3:26	1	OFF	CYCLE	0

21°C	21°C	21°C	21°C	WINDOW	3
95°C	56°C	72°C	6°C	PROG#	1
0:00	0:00	7:00	0:00	CYCLES	1
RUNTIME	3:26	1	OFF	CYCLE	0

21°C	21°C	21°C	21°C	WINDOW	4
95°C	56°C	72°C	6°C	PROG#	1
0:00	0:00	0:00	0:00	CYCLES	0
RUNTIME	3:26	1	OFF	CYCLE	0

TROUBLESHOOTING

Observations	Suggestions
Temperature cyler will not start	Switch in right rear of machine not switched to "ON" position or plug is loose. Ensure temperature cyler is plugged in and switch is in the "ON" position
	Blown fuse. Replace the fuse inside the power entry module located at the rear of the instrument
Program will not run, display returns to EDIT or EDIT/REVIEW mode.	Incorrect parameters programmed into system. Check all four windows of the program to make sure all valid parameters are programmed in
	Failure to push ENTER or RUN/STOP button. Pressing ENTER starts a reaction when blocks preheat to set temperatures. Pressing RUN/STOP can either begin a program immediately or automatically after blocks reach set temperatures
EXT FUNCT menu is not accessible	SHIFT key not pressed before the appropriate function key. To access special features in EXT FUNCT, press SHIFT key firmly (do not hold key down). When the SHIFT key is activated, the SHIFT prompt will appear in the lower right-hand corner of the screen. Then press the desired function key

MAINTENANCE

Note Before cleaning or performing maintenance unplug the temperature cycler and be sure the blocks have cooled to room temperature.

- ♦ Always operate the RoboCycler temperature cycler on a flat, stable surface.
- ♦ Blot up any oil spills. Wipe blocks periodically with a clean cloth or towel to maintain clean surfaces. Use an ammonia-based household cleaner for this purpose. Do not use alcohol or organic solvents on any part of the RoboCycler temperature cycler.
- ♦ Remove accumulated condensation from the cold block.
- ♦ Clean fan filter guard with warm soapy water. The outside guard snaps off for easy cleaning.

SPECIFICATIONS

RoboCycler Temperature Cycler ^a	
Block capacity RoboCycler® 40 temperature cycler RoboCycler® 96 temperature cycler	40 600 µl-tubes 96 200-µl tubes, 200-µl thin-wall tubes and /or strip tubes, or one 96-well polypropylene plate or polycarbonate plate
Temperature ranges Blocks 1,2,3 Block 4	25–99°C 6–25°C
Well-to-well block uniformity	±0.1°C, all blocks
Temperature control accuracy	± 0.5°C
Block heating speed	Approximately 5°C per minute.
Temperature sensors	Thermistors located inside each block
Number of programs	99 programs, 4 windows/program
Program linking	Up to 10 programs (40 windows) can be linked and run sequentially
Maximum cycles	99 cycles/window
Maximum segment time	99 minutes, 59 seconds
Dimensions	31 cm W × 49 cm D × 21 cm H
Power requirements	100–120 VAC, 4.0 A or 230 VAC, 2.0 A
Fuse	4.0 A/250 V Type T for 100- and 120-V operation 2.0 A/250 V Type T 230-V operation

^a See Appendices I and II for specifications of the Hot Top Assembly and the Gradient Feature.

APPENDIX I – HOT TOP ASSEMBLY AND OPERATION

Catalog #400891, #400893, #400897, #400898, and #400899

Introduction

The optional Hot Top Assembly for the RoboCycler temperature cyclers[†] eliminates the need to add an oil overlay to thin-wall reaction tubes by maintaining a temperature that reduces the condensation formed in the reaction tubes during temperature cycling.

Materials Provided With The Hot Top Assembly

Materials provided	Quantity
Hot Top Paddle assembly	1
Hardware	
0.25-inch × 0.62-inch cap-head screw	1 (Hot Top 40 only)
0.25-inch × 0.88-inch cap-head screw	1 (Hot Top 96 only)
Power cable	1
3/16-inch hex wrench	1
RoboCycler [®] temperature cyclers to Hot Top Assembly interface cable	1

Installation

The acrylic safety cover should already be installed. If it is not, install it before continuing (See *Installing the Safety Cover*).

1. Remove the standard paddle assembly from the temperature cyclers by removing the paddle screw (see Figure 1).
2. Attach and tighten the paddle assembly of the Hot Top Assembly with the provided cap-head screw using the 3/16-inch hex wrench.

Note *The alignment pin on the rotating shaft must be in the 12 o'clock position, viewed from the front of the temperature cyclers. Check the position of the alignment pin before positioning the paddle assembly on the rotating shaft. If the alignment pin needs to be repositioned to 12 o'clock, rotate the shaft by hand. Make sure the alignment pin on the rotating shaft is inserted into the alignment hole on the bottom surface of the paddle (See Figure 3).*

3. Connect the Hot Top Assembly coiled control cable to the Hot Top power entry module (See Figure 2). The power entry module is pre-installed on all RoboCycler temperature cyclers.

[†] See *Endnotes*.

4. Connect the RoboCycler interface cable between the temperature cycler back panel and the back of the Hot Top Assembly power entry module (located on top of the acrylic cover).
5. Plug the appropriate end of the power cord of the Hot Top Assembly into the back of the power entry module and the other end into a grounded power outlet.

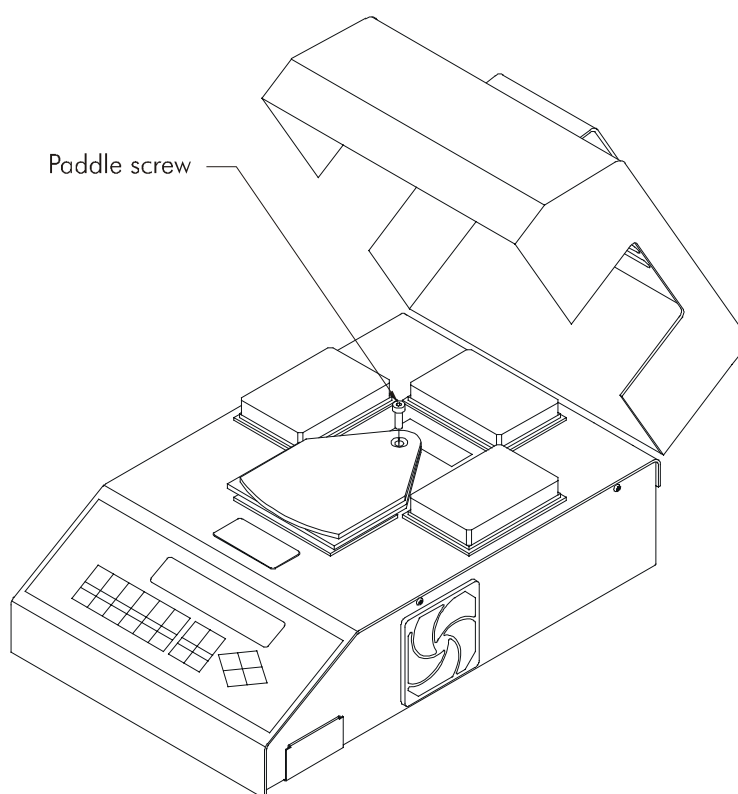


FIGURE 1 Standard paddle assembly of the RoboCycler temperature cycler.

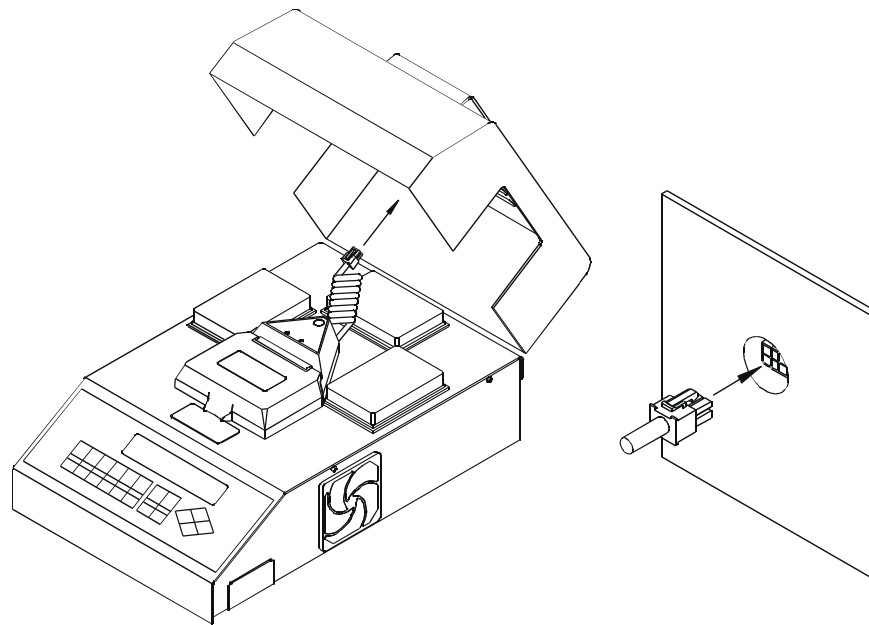


FIGURE 2 Connecting the Hot Top Assembly coiled control cable to the power entry module.

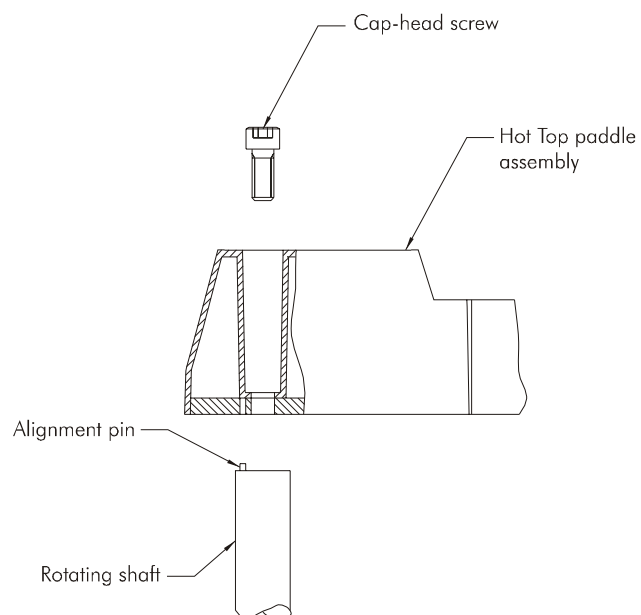


FIGURE 3 Installation of the Hot Top paddle assembly.

Position Calibration

Note *Position calibration is done during instrument manufacture. However, if the robotic arm is removed from the RoboCycler temperature cycler, the arm must be position-calibrated after being replaced on the machine. This adjustment is done automatically using the POSITION CALIBRATION function.*

1. Press *SHIFT* and then press *EXT FUNCT*.
2. Select *POS CALIBRATE (2)*.
3. Insert two tubes into the paddle while the paddle is positioned in Block 4 (the cold block).
4. Follow the directions indicated on the display. The robotic arm will lift and rotate, returning to rest at the cold block.
5. Press *ENTER* to complete the calibration routine. The system will return to Extended Functions menu. The robotic arm is now calibrated.
6. Exit the Extended Functions menu.

Preoperating Considerations

- ♦ Because the RoboCycler temperature cycler with the Hot Top Assembly heats tubes from both the top and the bottom, it may be necessary in some cases, after the Hot Top Assembly has been installed, to reoptimize PCR parameters that were determined before installation.
- ♦ It should be noted that while waiting to run, the heat from the Hot Top Assembly may affect the initial temperature of the cold block. It is recommended, therefore, that reactions be prepared on ice and then be placed into the Hot Top Assembly just prior to the start of the run.
- ♦ Use only thin-wall tubes with the Hot Top Assembly. Make sure the caps of the tubes are always securely fastened.
- ♦ Although the Hot Top Assembly significantly reduces condensation, a slight ring of condensation may appear above the samples during cycling. However, the resulting product will not be affected.

Temperature Limitations of the Hot Top Assembly

When configured for the Hot Top Assembly, the temperature gradient function of the RoboCycler Gradient temperature cycler is limited. See Appendix II for more information. Also, all non-gradient hot blocks are limited to a low temperature of 55°C.

Reaction Tubes Compatible with the Hot Top Assembly

The temperature cycler with the Hot Top Assembly is configured for single 600- μ l thin-wall tubes (RoboCycler 40 temperature cycler) or single 200- μ l, thin-wall tubes, 8- or 12-tube, thin-wall tube strips with caps, and 96-well, polypropylene plates with caps (RoboCycler 96 temperature cycler). Do not use polycarbonate 96-well plates or sealing tapes in a temperature cycler equipped with the Hot Top Assembly.

Operating Instructions

Initial Start-Up

Caution *To avoid injury, load and unload tubes carefully when the Hot Top Assembly is on.*

1. The Hot Top Assembly is automatically powered on when *RUN/STOP* is pressed to start the temperature cycler. The red indicator light on the paddle assembly illuminates, indicating that the unit has power (see Figure 4). When the green indicator light on the paddle assembly illuminates, the Hot Top Assembly is at the proper starting temperature and is ready to use (see Figure 4).

Note *The temperature cycler does not detect when the Hot Top Assembly is at the proper starting temperature. When the blocks begin heating from room temperature, the Hot Top Assembly takes less time to reach temperature than the blocks of the temperature cycler. Therefore, the Hot Top Assembly will have reached proper starting temperature by the time the temperature cycler has reached cycling temperature. The Hot Top Assembly can, however, be preheated by pressing *SHIFT*, then *EXT FUNCT*, then 5, or *SHIFT* then 5. This will turn the Hot Top Assembly on without starting the temperature cycler.*

2. The paddle assembly returns to the cold block following completion of cycling, shuts off automatically, and begins cooling. The Hot Top Assembly is automatically shut off if a running protocol is exited or terminated. The Hot Top Assembly can also be powered off and on during cycling by pressing *SHIFT* then 5.

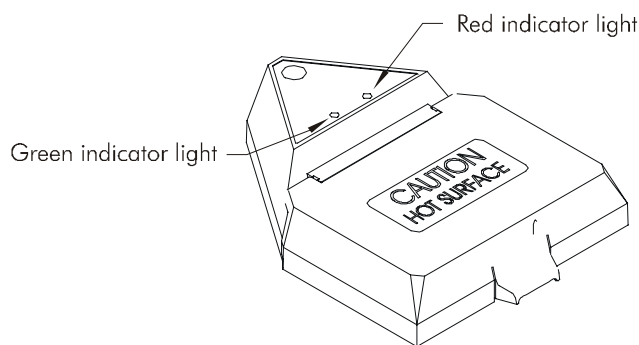


Figure 4 Indicator lights on the paddle assembly.

Hot Top Assembly Troubleshooting

Observations	Suggestions
The red indicator light is not illuminated	The power cord is not attached properly or is inserted loosely into the power source. Make sure the Hot Top Assembly is plugged securely into a power source
	The fuse at the rear of the power entry module is nonfunctional. Replace the fuse with a 5 × 20-mm, type-F, 0.8-A, 250-V fuse
	The interface cable is not attached properly. Make sure the interface cable is securely connected to the Hot Top Assembly and the RoboCycler base
Excessive condensation is observed	The Hot Top Assembly has been inadvertently turned off during cycling. Do not press Shift followed by 5 during the cycling run
Results differ from those obtained with the oil-overlay method	Heat emitted by the Hot Top Assembly may cause slight changes from the optimal PCR parameters determined with the standard paddle. Optimize PCR parameters for use with the Hot Top Assembly
Unable to program a 22°C-degree temperature gradient into the RoboCycler Gradient 96 temperature cycler	Use of the Hot Top Assembly limits the temperature gradient to a range of 11°C. Program a temperature gradient range of 11°C or select the standard paddle in Extended Functions (See Appendix II)

APPENDIX II - GRADIENT FEATURE

RoboCycler Gradient temperature cyclers are designed to allow the user to quickly find the correct annealing temperature for thermal cycling. By setting a linear temperature gradient across the gradient block, the user can quickly optimize annealing reaction conditions in one experiment. Block 2, the brass gradient block, allows simultaneous evaluation of up to 12 different annealing temperatures for cycling reactions (up to 8 for the RoboCycler Gradient 40 temperature cycler).

The system has preset gradient-temperature modes, or the user can create custom programs for any temperature gradient within a temperature range of 35° to 90°C (45° to 99° C for the RoboCycler 96 Gradient temperature cycler with the Hot Top Assembly installed). The temperature ranges of the RoboCycler Gradient temperature cyclers are shown in the table below. Once the optimum temperature is determined, the gradient block can be used in a standard single-temperature mode.

Gradient Temperature Ranges

RoboCycler model	Maximum gradient range
RoboCycler Gradient 40	
If low end of gradient range = 35–41°C	7°C
If low end of gradient range = 42–85°C	14°C
RoboCycler Gradient 96	
If low end of gradient range = 35–41°C	11°C
If low end of gradient range = 42–77°C	22°C
RoboCycler Gradient 96 with the Hot Top Assembly installed (low end of gradient range = 45–88°C)	11°C

Using the Gradient Feature

The gradient feature of the RoboCycler temperature cyclers is accessed through the *SHIFT* functions. To access the built-in special features of the RoboCycler Gradient temperature cyclers press the *SHIFT* key, then press the *SET GRAD* (1) or *CLR GRAD* (2) key (see Figure 2).

	BLOCK 1	BLOCK 2	BLOCK 3	BLOCK 4		
BLOCK TEMP	21°C	23° 23°	21°C	21°C	WINDOW 4	
SET TEMP	95°C	47° 58°C	25°C	6°C	PROG#	1
	04:30	05:00	10:00	00:00	CYCLES	1
	RUNTIME	00:00	1	ON OFF	CYCLE	1
TEMP MODE GRADIENT LINK PGM						

Figure 1 LCD window of a RoboCycler Gradient 96 temperature cycler in gradient mode. Note that Block 2 displays two temperatures corresponding to the temperatures in columns 1 and 12 of Block 2 (columns 1 and 8 on a RoboCycler Gradient 40 temperature cycler).

Set Gradient

To turn on the gradient feature, press *SHIFT*, then press *SET GRAD* (1). The Set Gradient menu appears as follows (note that this screen is for a RoboCycler Gradient 96 with the Hot Top Assembly installed):

SELECT GRADIENT TEMPERATURE RANGE	
[1] = 47–58°C	[4] = 61–72°C
[2] = 52–63°C	[5] = Custom Range
[3] = 56–67°C	[6] = Clear Gradient

Program the temperature cycler for the desired gradient mode by selecting one of the four preset gradient temperature ranges, or select the custom range option to create a custom gradient range.

Fixed Range Gradient Temperatures (Modes 1–4)

The fixed range gradient Modes 1–4 are preset temperature ranges that, when selected, program all four windows of the current program to that specific range.

Custom Range Gradient Temperature (Mode 5)

The custom range gradient temperature option (Mode 5) allows the user to manually enter the gradient-temperature range for each individual window of the current program. Selecting Mode 5 initially sets the gradient high and low temperatures in all windows to 35°C (45°C for the RoboCycler Gradient 96 temperature cycler with the Hot Top Assembly installed), and allows the user to define the gradient range by using the arrow keys or the *ENTER* key to position the cursor in the desired gradient high- or low-temperature field, and then enter the desired temperature.

Note *Exercise caution in Custom Range Gradient Mode 5 when programming in different gradient temperature ranges for each window. Be sure to allow the RoboCycler Gradient temperature cycler the time to adjust to the different ranges (See Smart Programming).*

An error message will display when *RUN/STOP* is pressed if the temperature range setting is exceeded or if the low-temperature setting exceeds the high-temperature setting.

Note *The gradient ranges for temperature cyclers with Hot Top Assemblies installed are limited not by the physical installation of the Hot Top Assembly but by having the Hot Top paddle selected as the paddle type in the Extended Functions menu (See Extended Functions, Paddle Type). If the Hot Top Assembly is installed but the standard paddle assembly is selected in the software, the Hot Top Assembly will still function and a greater temperature gradient can be achieved. In this case, however, Stratagene does not guarantee the linearity of temperature of a gradient range which is greater than 11°C on a RoboCycler Gradient 96 temperature cycler with the Hot Top Assembly installed.*

Clear Gradient

Clear Gradient (6) resets the gradient temperature settings in all four windows of the current program to a steady state, nongradient temperature setting (See *Clear Gradient*). The display will prompt the user to *KEEP GRADIENT MODE (1)* or *CLEAR GRADIENT MODE (2)* for all windows in the program.

The gradient settings in all four windows of the current program can be cleared to a nongradient temperature setting by pressing *SHIFT* then *CLR GRAD*. When clearing a gradient setting, all Block 2 temperatures will default to 25°C.



Sample RoboCycler Gradient PCR Program

The RoboCycler temperature cycler saves experimental time by moving samples from one preequilibrated block to another. The first sample below, *Sample RoboCycler Gradient PCR Parameters*, illustrates an example of thermal cycling parameters for PCR. The second sample, *Sample RoboCycler Gradient PCR Program Entry* shows how to program these parameters into the RoboCycler temperature cycler.

Sample RoboCycler Gradient PCR Parameters^a

Window	Block 1	Block 2 (Gradient)	Block 3	Cycles
1	95°C/ 1:00 min.	—	—	1
2	95°C/1:00 min.	44–66°C/ 1:00 min.	72°C/3:00 min.	30
3	—	—	72°C/7:00 min.	1
4	—	—	—	—

^a At the end of the program, the tubes will automatically hold in the cold block at 6°C until *Enter* is pressed.

Sample RoboCycler Gradient PCR Program Entry

21°C	21°	21°C	21°C	21°C	WINDOW	1
95°C	44°	66°C	72°C	6°C	PROG#	1
1:00	0:00	0:00	0:00	0:00	CYCLES	1
RUNTIME	3:26	1	ON	OFF	CYCLE	0

21°C	21°	21°C	21°C	21°C	WINDOW	2
95°C	44°	66°C	72°C	6°C	PROG#	1
1:00	1:00	3:00	0:00	0:00	CYCLES	30
RUNTIME	3:26	1	ON	OFF	CYCLE	0

21°C	21°	21°C	21°C	21°C	WINDOW	3
95°C	44°	66°C	72°C	6°C	PROG#	1
0:00	0:00	7:00	0:00	0:00	CYCLES	1
RUNTIME	3:26	1	ON	OFF	CYCLE	0

21°C	21°	21°C	21°C	21°C	WINDOW	4
95°C	44°	66°C	72°C	6°C	PROG#	1
0:00	0:00	0:00	0:00	0:00	CYCLES	0
RUNTIME	3:26	1	ON	OFF	CYCLE	0

RoboCycler Gradient Thermal Cycler Specifications

Gradient Feature	Specification
Temperature range RoboCycler Gradient 96 temperature cycler RoboCycler Gradient 40 temperature cycler	Configured for Hot Top Assembly: 45–99°C, 11°C gradient range Configured for standard paddle: 35–99°C: Low-end temp 35–41°C, maximum gradient range is 11°C Low-end temp ≥42°C, maximum gradient range is 22°C 35–99°C Low-end temp 35–41°C, maximum gradient range is 7°C Low-end temp ≥42°C, maximum gradient range is 14°C
Well-to-well block uniformity	Gradient block: ±0.25°C (Block 2)
Temperature control accuracy	± 0.5°C
Temperature sensors	Thermistors located inside block
Gradient programs	4 preset modes or custom-programmed by user

APPENDIX III - THERMAL TRANSFER BLOCK

Catalog #400834

The Thermal Transfer Block is a convenient hot or cold dry bath designed for use with RoboCycler temperature cyclers.⁴ The temperature transfer block is placed on top of a hot block, the gradient block, or the cold block (see Figure 1). The robotic arm is not used when temperature transfer blocks are in place.

The transfer block has 38 wells: 24 wells for 1.5-ml tubes and 14 wells for 500- μ l tubes. The block's center well is a temperature well for a conventional glass bulb thermometer, to allow measurement of the temperature of the transfer block. A peripheral insulation jacket allows safe, convenient transfer of the block from the RoboCycler temperature cycler to the laboratory bench.

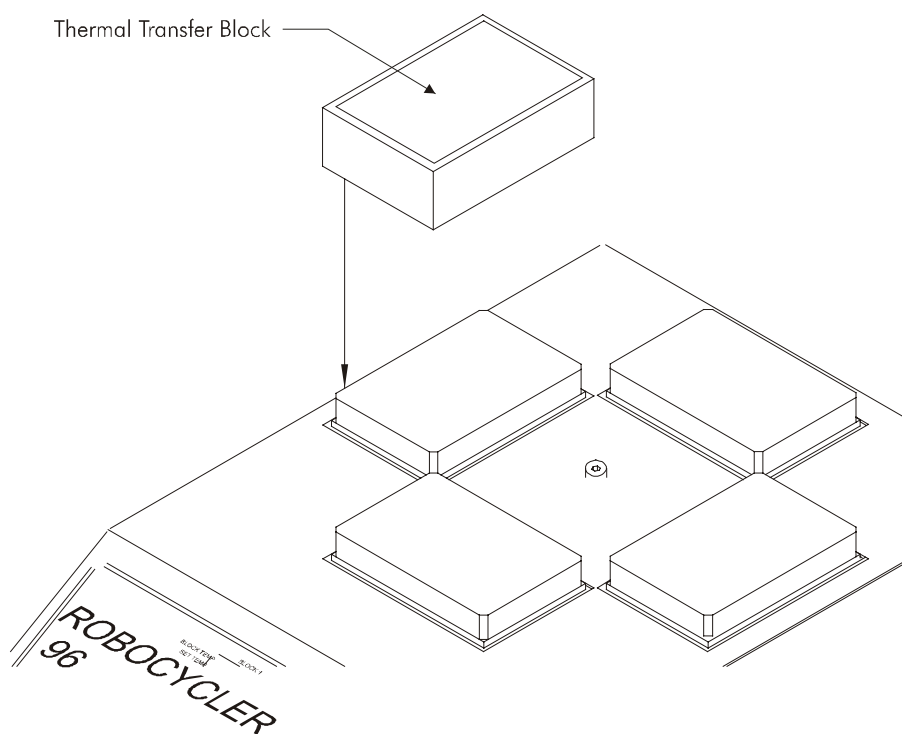


FIGURE 1 Thermal Transfer Block.

Protocol

Note *If the RoboCycler thermal cycler is equipped with a gradient feature, **do not** use a Thermal Transfer Block with Block 2 (the gradient block) in gradient mode. The Thermal Transfer Block can be used on the gradient block if the gradient block is set in a standard single-temperature mode.*

1. Turn on the power switch located in the right rear of the RoboCycler temperature cycler.

Note *The paddle arm will make one quick rotation to ensure that the arm will start at the home position, the cold block (Block 4).*

2. Place the Thermal Transfer Block on top of the temperature cycler block. Up to three Thermal Transfer Blocks, one per block of the temperature cycler, except where the paddle is, can be used. If the Thermal Transfer Block is used on the cold block, gently lift the paddle and turn it to another block.
3. Using the control pad on the RoboCycler temperature cycler, enter the temperature desired for the block.
 - ♦ Select the *SHIFT* key then *EXT FUNCT*.
 - ♦ Select 1) Set Temp Run mode
 - ♦ Select 2) Begin Running at TEMP with ENTER
 - ♦ Select 7) Exit and type in the temperature desired
 - ♦ Select RUN/STOP

The RoboCycler temperature cycler will begin heating the blocks. Once the blocks preheat to the set temperatures, the temperature transfer block may be removed if desired. **Do not press ENTER until the Thermal Transfer Block is removed**, because pressing *ENTER* starts a program and causes the robotic arm to rotate.

Note *The time required to heat the Thermal Transfer Blocks will vary depending on the ambient temperature. On average, the blocks will reach temperature in 15 to 30 minutes.*

Maintenance

Care should be taken to keep the bottom of the Thermal Transfer Block clean and free of nicks, which will decrease the temperature transfer efficiency.

Thermal Transfer Block Specifications

Feature	Specification
Size	12.0 cm L × 8.3 cm W × 3.8 H cm
Weight	0.6 kg
Well Specifications	24 1.5-ml wells 14 500-μl wells 1 thermometer well

REFERENCES

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4. Danssaert, J., Hayfield, J., Schoettlin, W. and Monaco, M. (1993) *Strategies* 6(1):30.

ENDNOTES

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† Purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (“PCR”) process for research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased (i.e., an Authorized Thermal Cycler).

†† These products are optimized for use in the Polymerase Chain Reaction (“PCR”) process covered by patents owned by Hoffmann-La Roche. Purchase of these PCR-related products does not convey any rights under the PCR patents owned by Hoffmann-La Roche. A license to use the PCR process accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.

††† Practice of the patented Polymerase Chain Reaction (PCR) process requires a license. Stratagene’s temperature cycler is an Authorized Temperature cycler. Its use with Authorized Reagents provides a limited PCR license in accordance with the label rights accompanying such reagents. It may also be used with PCR licenses available from The Perkin-Elmer Corporation.



ROBOCYCLER PROGRAMMING WORKSHEET

Window 1

Cycles _____

Block #	1	2	3	4
Temperature				
Time				

Window 2

Cycles _____

Block #	1	2	3	4
Temperature				
Time				

Window 3

Cycles _____

Block #	1	2	3	4
Temperature				
Time				

Window 4

Cycles _____

Block #	1	2	3	4
Temperature				
Time				



ROBOCYCLER TEMPERATURE CYCLER QUICK-REFERENCE COMMAND LIST

Feature	Keystroke Sequence
Set Gradient	SHIFT + SET GRAD
Clear Gradient	SHIFT + CLR GRAD
Set Time	SHIFT + SET TIME
Set Date	SHIFT + SET DATE
Store Program	SHIFT + STORE PGM
Recall Program	SHIFT + RECALL PGM
Run Status	SHIFT + RUN STATUS
Extended Functions	SHIFT + EXT FUNCT
Clear Window	SHIFT + CLR WINDOW
Copy Window	SHIFT + NEXT WINDOW
Raise Paddle/Lower Paddle	SHIFT + ^ / SHIFT + v
Toggle Hot Top Assembly Power On/Off	SHIFT + 5
Reset Serial Communications	SHIFT + EXT FUNCT + CLEAR
Temp Run Mode	SHIFT + EXT FUNCT + 1
Position Calibrate	SHIFT + EXT FUNCT + 2
Link Programs	SHIFT + EXT FUNCT + 3
Beep On/Off	SHIFT + EXT FUNCT + 4
Hot Top On/Off	SHIFT + EXT FUNCT + 5
Paddle Type	SHIFT + EXT FUNCT + 6