**MyCycler™ Thermal Cycler**

**User Guide**

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Table of Contents

[I. Disclaimer! 2](#_Toc429656553)

[II. Safety Considerations 2](#_Toc429656554)

[III. The Polymerase Chain Reaction 2](#_Toc429656555)

[IV. Operation of the MyCycler 2](#_Toc429656556)

[A. Turning on the MyCycler 2](#_Toc429656557)

[B. Creating New Protocols (Basic Options) 2](#_Toc429656558)

[*i.* *Soft Key Functions for the Edit Screen* 3](#_Toc429656559)

[*ii.* *Specifying Time Values* 3](#_Toc429656560)

[*iii.* *Specifying Dwell Time* 3](#_Toc429656561)

[*iv.* *Specifying Temperature Values* 4](#_Toc429656562)

[*v.* *Specifying Temperature Gradients* 4](#_Toc429656563)

[*vi.* *Changing the Number of Cycles or Steps* 4](#_Toc429656564)

[*vii.* *Completing Protocols* 4](#_Toc429656565)

[*viii.* *Naming Protocols* 5](#_Toc429656566)

[C. Editing Existing Protocols 5](#_Toc429656567)

[D. Running Protocols 5](#_Toc429656568)

[*i.* *Run Screen* 6](#_Toc429656569)

[*ii.* *Pausing a Running Protocol* 6](#_Toc429656570)

[*iii.* *Terminating a Running Protocol* 6](#_Toc429656571)

[*iv.* *A Power Failure while a Protocol is Running* 7](#_Toc429656572)

[E. End of Run Options 7](#_Toc429656573)

[*i.* *End of Run Screen* 7](#_Toc429656574)

[*ii.* *The Run Report* 7](#_Toc429656575)

## Disclaimer!

Please note: this is an abbreviated guide. The actual manual for this instrument is 38 pages. While it is a good exercise for any student to read the user manual when beginning work on a new instrument, this User Guide can serve as a guide to general use of the instrument and may be sufficient for *many uses*. However, in the event that you encounter trouble, need more information, or require assistance, contact Amy (email: areplogle@pugetsound.edu; location: Room 117H, Thompson Hall; phone: 253-879-2829) or refer to the manual for the instrument.

## Safety Considerations

The sample block of the MyCycler thermal cycler can reach temperatures of 100°C, and the heated lid can be programmed to maintain temperatures up to 110°C. The heated lid should remain closed at all times during operation to prevent accidental skin burns. Always allow the sample block to return to its idle temperature before opening the lid and removing samples. When placing or removing samples, open the heated lid all the way up to allow maximum clearance and to avoid accidental skin burns.

## The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a technology in molecular biology used to produce millions of copies of a specific DNA sequence from a mixture of DNA molecules in a relatively short amount of time. The PCR machine increases and decreases the temperature of the sample in automatic, programmed steps, to complete the synthesis of a specific DNA sequence.

## Operation of the MyCycler

### Turning on the MyCycler

* 1. Power up the MyCycler by pressing the **Standby** key on the front panel of the instrument. After a quick diagnostic routine, the **Home** screen will be displayed and you can proceed to one of the options detailed throughout this user guide.

### Creating New Protocols (Basic Options)

* 1. To create a new protocol press **F2-Create** from the Home Screen. You will be asked to select whether you wish to:
     1. Create a **Custom** protocol that is a completely new protocol you will define. If you choose this option, the default custom protocol is a simple three-step amplification protocol with one cycle; 30 seconds at 95°C, 30 seconds at 55°C, followed by 0 seconds at 72°C.
     2. Use one of the Bio-Rad protocols as a template. Some protocol names end with a 2 and others end with a 3; the number indicates the number of steps in the amplification cycle of the protocol.

For either option you choose, you will need to enter values for time, temperature, and repeats, as described in the following sections. All protocols will have default values in each field. If you want to accept the value, press **Enter** or an arrow key. The **Clear** key may be used to remove all numbers in the time field.

* 1. Edit the fields of the protocol.

### *Soft Key Functions for the Edit Screen*

|  |  |  |
| --- | --- | --- |
| Softkey | Option | Description |
| F1 | <<Cycle | Shifts the protocol display to the left by one cycle |
| F2 | >>Cycle | Shifts the protocol display to the right by one cycle |
| F3 | Option | Adds or removes increments or decrements (see Advanced Options in this User Guide) |
| F4 | Add/Del | Adds or remove cycles and steps |
| F5 | Done | Allows you to save, revisit, or reject changes made to the protocol. Also allows you to run the protocol without saving changes. |

### *Specifying Time Values*

Time values may fall between 1 second and 99 minutes-59 seconds. You must use the mm:ss format (e.g., 00:01 to 99:59.) When you input a value, the numbers will be entered from right to left with implied zeros in the front. For example, to enter 1 minute 15 seconds, press 1, 1, 5 followed by the enter or an arrow key. **Note**: entering a fifth digit will cause the left-most digit to be lost.

### *Specifying Dwell Time*

Finite dwell times may be as long as 99 minutes and 59 seconds (99:59), or may be set as short as 0 seconds (00:00).

When the dwell time is set to 00:00, the MyCycler will heat or cool until it attains the **set point temperature** and then immediately begin heating or cooling to the next set point temperature.

Infinite dwell times may be programmed for any steps belonging to a cycle with only one repeat. When an infinite dwell time is reached, the instrument maintains the specified temperature until you intervene. If the infinite dwell time is programmed for any other step than the last step of the protocol, the instrument goes into **Pause** mode when it reaches that step, and holds the set point temperature until you press the **Resume Run** or **End Run** soft key. You may specify an infinite dwell time by pressing the **Shift** key, followed by the **0** key.

### *Specifying Temperature Values*

Temperature values must be in the range of 4.0°C to 100°C. Numbers are displayed on the screen as the keys are touched, beginning with the position immediately to the left of the decimal point, and then move to the left with each subsequent entry. No decimal point will be displayed until you press the decimal point key, **Enter** key, or one of the arrow keys. If a decimal point is not pressed, then a decimal point and 0 are appended to the temperature automatically. For example, to enter 95.0°C, press 9, then 5 and an arrow or **Enter** key. The display will show 95.0°C after the last key stroke. To enter 95.5°C, press 9, 5, decimal key, 5.

### *Specifying Temperature Gradients*

The MyCycler Thermal Cycler offers a temperature gradient option for optimization of assay conditions. Gradients may be programmed at any step of any cycle of the protocol. The gradient option allows input of a temperature gradient of 1-25 degrees in total temperature span at temperatures as low as 30°C and as high as 99°C.

Thermal Gradients in the MyCycler are formed from the front to the rear of the sample block resulting in eight specific temperatures for assay optimization.

To add a temperature gradient to your set-point temperatures, put the cursor on the desired temperature step of the protocol and press **F3-option**.

* From the menu, select **Temp Gradient**.
* Once selected, a default value of 1°C minimum-maximum gradient will be displayed. Input the desired range of the temperature gradient. The display will be updated with the temperatures for each row of samples.

### *Changing the Number of Cycles or Steps*

The **Add/Del** soft key (**F4**) allows you to change the number of cycles or steps. When **F4** is pressed, a menu of editing choices is displayed: **Add cycle before**, **Add cycle after**, **Add step before**, **Delete step**, and **Delete cycle**. Steps and cycles are added before or after, relative to the current cursor position. If **Delete step** or **Delete cycle** is chosen, the step with the cursor, or cycle with the cursor is deleted.

### *Completing Protocols*

When you have finished editing the protocol, press **F5-Done** to display a menu of options. The choices are:

* **Run Protocol**: To run the protocol immediately, without first saving it.
* **Save Protocol**: To save changes made to an existing protocol. Note: This option is unavailable when editing a Bio-Rad template protocol.
* **Save Protocol as…**: You will be asked to create a name for the protocol. See next section for naming instructions.
* **Abandon Edits**: Reverts the protocol without saving changes.
* **Resume Editing**: Allowed you to return to the protocol edit screen to make further changes.

### *Naming Protocols*

A protocol is given a name with selecting the **Save Protocol as…** option or the **Rename Protocol** option. Protocols are named using the alphanumeric keys. A new protocol is saved as a file under the **My protocols** column of the **Protocol Library**. Whenever the protocol is run, the name of the protocol appears on the **Run**. All protocol file names must be unique.

Centered below the display are ten alphanumeric keys (keys 0-9), which are arranged like the keys on a telephone. Each numeric key (except 1 and 0) is used to represent three or more letters.

When entering a protocol or instrument name into a screen display, the keys automatically shift to alpha more for the entry of letters. The word ALPHA is displayed in the top right corner of the screen. You can toggle between alpha and numeric by pressing the **Shift** key. To enter uppercase letters, press **F2-Caps Lock** before pressing the alphanumeric key.

To specify an alphabetic character, press the key until the desired character is displayed. For example the **2** key is used for a, b, or c entries. To input the letter a, press the **2** key once. To input b, press the **2** key twice and to input c, press the **2** key three times. If the **2** key is pressed a fourth time, it will display the letter a.

### Editing Existing Protocols

To edit an existing protocol, press **F1** from the **Home** screen. You may then select the desired protocol from the **Protocol Library**, and then select the **Edit Protocol** option. Follow the instructions in section B above to change the temperature, time, cycle, or step as needed.

### Running Protocols

To run a stored protocol:

* 1. From the Home screen press **F1** to display the Protocol Library.
  2. Use the arrow keys to highlight the desired protocol.
  3. Press **Enter** and from the selection box, choose **Run Protocol** and press **Enter**.
  4. Confirm the selections in the Run Setup screen.
     1. **Hot Start and the hot start temperature**: If **Hot Start** is chosen, the MyCycler will heat to the hot start temperature when the run is initiated and then go into Pause Mode.
     2. **Temperature measurement mode**: This is the method of temperature measurement. You can choose to monitor the block temperature of the reaction module or the sample temperature by algorithm. If you choose the Algorithmic method, then you must specify the volume of the sample (**Sample volume**).
  5. When the Run Setup options are specified, press **F5-Begin Run**. Protocol execution will begin as soon as the heated lid reaches the appropriate temperature.

### *Run Screen*

The Run screen will be displayed as the execution of the protocol begins. The Run screen displays the graphical representation of the protocol, with the set point temperature and dwell time of each step displayed on the graph.

The screen also shows the current temperature of the reaction module block, or the calculated sample temperature. The current repeat number and dwell time of the current repeat will also be shown, and will count down as the protocol progresses.

The name of the protocol is displayed to the right of the word **RUNNING**. If the protocol is an unsaved modification of a saved protocol, then the name of the saved protocol is displayed and preceded by the letter M (for modification) in parentheses.

The current time of day will be displayed along with the time the protocol was initiated, and with an estimated time of completion.

### *Pausing a Running Protocol*

When the **STOP** key or the **F1-Pause** key is pressed while a protocol is running, the MyCycler will go into Pause mode and the temperature of the reaction module block will be brought to the next set point temperature and held there. If the MyCycler is counting down a dwell time when the **STOP** or **F1-Pause** key is pressed, it maintains the temperature and the clock stops. If you choose:

* **F1-Resume Run**: The clock will immediately begin counting down the dwell time or resume counting down at the point at which the protocol was paused.
* **F2-End Run**: The instrument will be heated or cooled to the idle temperature and you will return to the Home screen.

### *Terminating a Running Protocol*

To terminate execution of a protocol before it has been completed, go into Pause mode, as described above, and select **F2-End Run** or press the **STOP** key.

### *A Power Failure while a Protocol is Running*

If a running protocol is interrupted by a power failure, the MyCycler will resume execution of the protocol automatically when power is restored. The protocol will resume at the step with the highest set point temperature of the cycle that was interrupted, or the same repeat that was interrupted. Each power failure is counted as an error at the end of the run, and is described in the Validation and Run reports.

If power is lost more than twice, the MyCycler will not resume execution of the protocol. If any single power interruption lasts longer than ten minutes, the protocol will not resume.

### End of Run Options

### *End of Run Screen*

The End of Run screen is automatically displayed after protocol execution. If will offer soft key options for getting reports on the protocol. If there was any problem with the execution of the protocol, a message to that effect will be displayed along with the appropriate instructions.

### *The Run Report*

Reports are textual summaries that record the details and settings of protocols run on the MyCycler. These reports are stored in the instrument memory for 30 days and cannot be deleted. Following the end of every protocol, both a **Validation Report** and a **Run Report** are made available by pressing **F2-Run Report** or **F3-Validation Report** from the **End of Run** screen. Furthermore, you may also access an index of every protocol run for the past 30 days from the **View Reports** option in the **Utilities** menu.

The **Run Report** is a subset of the information contained in the **Validation Report**. The Run Report includes the settings of the instrument and protocol, but does not track the execution of every step, cycle, and repeat of the protocol.

The **Validation Report** includes all the details of the Run Report, but also includes a list of every step, cycle, and repeat of the protocol, flagged as OK, Exceptions, or Errors, as follows:

* **OK**: Signifies that the instrument successfully attained the correct set point temperature, and held it for the correct about of time. The step will have an “OK” appended to it.
* Exceptions and Errors: Signifies that an exception or error has occurred, or the user as intervened with the progress of the protocol. Exceptions and Errors are denoted in the Validation Report with a “\*\*” flag.