

Stratagene Gradient Cycler

G5100A with 96-well thermal block

**G5100B with 96-well fast thermal
block**

G5100C with 384-well thermal block

User Guide

Version D, December 2008



Agilent Technologies

Notices

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CAUTION

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In this Guide...

This document describes how to program and use the Stratagene Gradient Cycler.

If you have comments about this protocol, send an e-mail to feedback_lcms@agilent.com.

1 Before You Begin

This chapter contains information for you to read and understand before you start.

2 Getting Started

This chapter contains instructions to load samples and to get started with the software.

3 PCR Programs

This chapter contains instructions to work with PCR programs.

4 System Management

This chapter contains instructions to set up user accounts and cycler settings.

5 Installation and Maintenance

This chapter contains installation and maintenance instructions.

6 Reference

This chapter contains reference information for using your thermal cycler.

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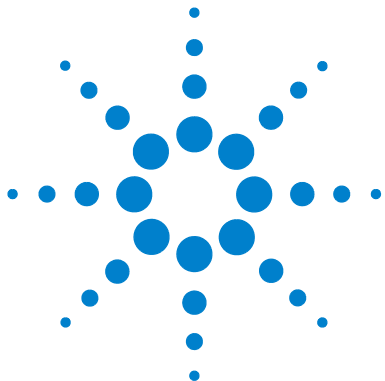
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This chapter contains information for you to read and understand before you start.

For installation instructions, see “[Installation](#)” on page 46.

The instructions in this guide apply to:

- G5100A Stratagene Gradient Cycler with 96-well thermal block
- G5100B Stratagene Gradient Cycler with 96-well fast thermal block
- G5100C Stratagene Gradient Cycler with 384-well thermal block



Materials Provided

Table 1 Materials Provided

Part	Quantity
Stratagene Gradient Cycler instrument	1
Thermal block	1
Power cord	1
Instruction manual	1

Safety Precautions

Electrical

Standard electrical safety precautions should be applied, including the following:

- Always put the instrument in a location where, if needed, the power supply can be immediately disconnected.
- Proper voltage must be supplied before you turn on the instrument for the first time.
- The device must be connected to a grounded socket.
- Do not touch any switches or outlets with wet hands.
- Turn off the instrument before you disconnect the power cord.
- Unplug the instrument before you clean any major liquid spills and before you service any of the electrical or internal components.
- Do not operate the instrument from a power outlet that has no ground connection.
- *Do not service the electrical components unless you are qualified to do so.*

Pinching Hazard

The lid presents a potential pinching hazard. Do not place hands or fingers into the cycler when the lid is being opened or closed.

Fluids and Reagents

- Fill reaction vessels outside the cyclers so that no fluids penetrate the instrument.
- Never cycle or incubate explosive, flammable and reactive substances in the thermal cycler.
- You must observe the relevant safety regulations when handling pathogenic material, radioactive substances or other substances hazardous to health.
- Do not submerge the instrument in any liquid.

Danger of Burns

- *Do not touch the thermal block, inner side of heated lid and reaction vessels.* These areas quickly attain temperatures of greater than 50°C. Keep the heated lid closed until temperatures of 30°C or lower are reached.
- Do not use any materials (plates, sealings, foils, mats) which are not sufficiently temperature-stable (up to 120°C).

Operating Environment

- The ventilation slots of the device must remain free to vent at all times. Leave at least 10 cm of space around the thermal cycler.
- Keep the ambient temperature between 10°C and 30°C with humidity levels between 0% and 95%.
- Do not operate the thermal cycler in a hazardous or potentially explosive environment.

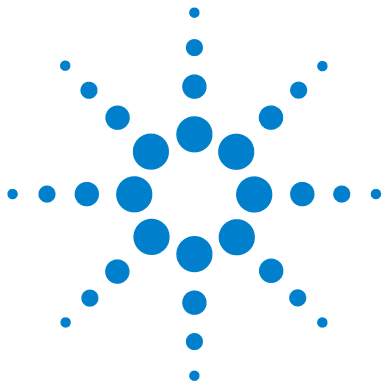
Overview

The Stratagene Gradient Cycler is designed to perform polymerase chain reaction (PCR) and related methods for amplification of DNA templates. The cycler can run even the most complex thermal cycling techniques, including time and temperature increments, touchdown PCR and temperature gradients. The software interface, operated through the color touchscreen, features an intelligent Program Wizard that can create PCR protocols automatically from primer and template information. The software also comes preloaded with optimized protocols designed for use with a variety of Stratagene PCR enzymes and kits.

Three different interchangeable thermal blocks can be used with the instrument: a 96-well thermal block, a 384-well thermal block, and a 96-well fast-ramping thermal block. Because the blocks are interchangeable, additional thermal blocks may be purchased separately and exchanged in the instrument as needed (see table below for a list of part numbers). This flexibility allows you to select the most suitable block for each PCR run based on the format needs and cycling speed requirements of the experiment. All three blocks are capable of holding a temperature gradient, and the fast-ramping block features a ramping rate of 5°C per second for heating and 3.5°C per second for cooling. No tools are required to exchange the block and the process takes only seconds to perform.

Table 2 Interchangeable Thermal Blocks

Thermal Block	Part Number
96-well thermal block	G5100-64001
96-well fast thermal block	G5100-64002
384-well thermal block	G5100-64003



2 Getting Started

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This chapter contains instructions to load samples and to get started with the software.



Sample Loading

To prevent damage to the block and the heated lid make sure you use only recommended sample tubes and plates listed in “[Compatible Plasticware Formats](#)” on page 54. Unsuitable tubes/plates can become damaged during cycling, which causes the escape of sample material.

WARNING

Danger of Burns: The thermal block, sample tubes and plates may reach temperatures as high as 100°C. Before loading or unloading the cycler, keep hands away until temperature reaches 30°C or less

To load plates

Depending upon the thermal block installed, the thermal cycler can be loaded with one 96-well PCR plate or one 384-well PCR plate.

- 1 Put your plate on the block.
- 2 Check that it is correctly positioned in the provided space to prevent damage to the block and the lid.

To load tubes

Stratagene gradient cyclers can be loaded with up to 96 PCR tubes (0.2 ml) or 12 strip tubes when using the 96-well block.

- Distribute tubes equally over the block.
- If your PCR program includes a Gradient step, load the sample tubes into the central rows of the thermal block (rows D and E).

To set the Lid Pressure

The lid on the Stratagene gradient cycler opens and closes manually.

1 Close the lid and turn the knob.

This sets the pressure to low pressure for tubes. If high pressure for plates is required, turn the knob slightly further. Always ensure that the heated lid is closed when heating tubes in the block.

Starting Up the Software

Touchscreen Operation of the Software

The color touchscreen allows you to operate the software by touching the buttons on the screen. If a mouse has been connected to the instrument, you may select buttons by clicking. When software functions require data input from a keyboard, the software automatically displays a virtual keyboard window operated through the touchscreen. Data may also be entered using an externally connected keyboard.

Start Screen

Shortly after turning on the instrument, the touchscreen displays the Start Screen. This screen is the starting point for all the software-controlled instrument operations as well as file and account management. Each navigation button on the Start screen menu takes you to a different functional area in the software.

Start Screen Menu

The table below describes the function associated with each navigation button on the Start screen:

Table 3 Start Screen Button Descriptions

Button	Description
New Program	Use this button to create a new PCR program.
Load/Edit	Opens the file directory to browse stored PCR programs. Use this button to edit or run programs.
Program Wizard	Use this button to create a new PCR program using a guided Program Wizard.
Start	Opens the file directory to browse stored PCR programs. Use this button to start a PCR program.
Incubate	Starts a single temperature incubation for a predefined period or for an indefinite hold.
Login	Opens the Login screen.

Table 3 Start Screen Button Descriptions (continued)

Button	Description
Reports	Opens the report directory allowing you to browse for stored GLP and LabBooks reports.
Settings	Use this button to adjust cyclers settings and manage user accounts.

User Levels

Three different user levels are available: Administrator, Registered user, and Guest user. Each level has specific user rights. Only administrators are authorized to set up new users and to change and/or assign user rights and passwords.

Table 4 Access levels

User Level	Functions allowed
Guest User	<ul style="list-style-type: none"> • Access rights to the Guest folder • Creating, copying, editing and executing programs stored in the Guest folder
Registered User	<ul style="list-style-type: none"> • Access rights to the Guest and personal folders • Creating, copying, editing and executing programs stored in the Guest and personal folders
Administrator	<ul style="list-style-type: none"> • Access rights to all Guest and user folders • Setup and maintenance of user accounts • Setup and maintenance of cyclers • Archiving/maintenance of all reports

To log in as Guest

- No password is required to login as *Guest*. By default, the *Guest* user account is automatically logged into the system each time the instrument is turned on.

When logged in as *Guest*, you have restricted user rights and work from the unprotected Guest folder, which can be accessed by all users. Before you can operate the thermal cycler as a registered user, an administrator has to set up your new user account. Once you are a registered user, you have a personal, encrypted file folder where you can manage your own PCR program files.

To log in as Administrator

- 1 From the Start screen, click the **Login** button by either touching the screen or clicking with a mouse.
The Login screen appears.
- 2 Select **Administrator** from the **Username** drop-down menu and enter the password. The default Administrator password is `admin`.
- 3 Click or touch **Login**.
You are now logged in to the Administrator account.

CAUTION

Change the factory-set login password to prevent unauthorized access to the instrument.

To log in as a Registered User

- 1 “[To add new user accounts](#)” on page 42 From the Start screen, click or touch **Login**.

The Login screen appears.

- 2 Select the user name from the **Username** drop-down list and type the password.
- 3 Click or touch **Login**.

This logs you into the system and gives you reading and writing rights as specified for your user level.

An administrator can reset user passwords if needed. See “[To edit user accounts](#)” on page 43.

To change users

No logout process is required to change which user is logged in.

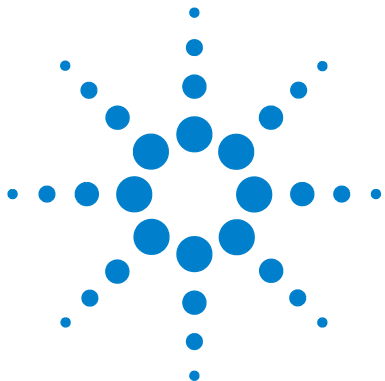
- 1 Click or touch **Login** from the Start screen.
- 2 Select the new user from the **Username** drop-down menu.
- 3 If logging in with a registered user name or as Administrator, type the password for that account.

If logging in as Guest, leave the Password text box blank.

- 4 Click or touch the **Login** button.

A confirmation message appears to verify the name of the user logged in to the system.

2 Getting Started
To change users



3 PCR Programs

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This chapter contains instructions to work with PCR programs.



Creating New PCR Programs

New PCR programs can be created in one of two ways: by manually creating a customized program step by step using a list of available commands, or by using the Program Wizard to generate the program automatically. The two methods are described in the following sections.

To manually create programs

Use the following steps to manually generate a new PCR program. For a detailed description of each command, see [“Program Steps in the Commands List”](#) on page 34.

- 1 Select **New Program** from the Start screen. The Compose a New PCR Program screen opens, which lists available drag-and-drop functions.
- 2 From the available program steps listed in the **Command List** on the right side of the screen, determine the order of steps that will make up the new PCR program.


See [“Program Steps in the Commands List”](#) on page 34.

- 3 Use the mouse or touchscreen to drag and drop the first step of the program from the *Command List* into the *Program Window* box.

This opens a dialog window for the selected step.

- 4 Enter the necessary settings for the newly added step as prompted.
- 5 Confirm your settings by clicking or touching on **OK**. You are returned to the Compose a New PCR Program screen.
- 6 Repeat [step 3](#) through [step 5](#) for each program command to be added to the program.
- 7 When the program is finished, click or touch **Save As** to save the file.
- 8 Type in a file name and click **Save**.
- 9 To start the program:
 - a Select **Run**.
 - b Enter the reaction volume when prompted.

[“Running a PCR Program”](#) on page 28.

You can change the command order at any time. Just drag the selected command to a new position within the Program Window. To delete a program step, drag it to the trash bin icon. 

Follow these guidelines when you create PCR programs:

- Cycles cannot be nested. A Start Cycle command must be followed by an End Cycle command before the next Start Cycle command.
- A Barcode Input step cannot be programmed inside a cycle.
- Only one Program Information step may be added to a program.
- The maximum number of Temperature and Gradient steps inside a cycle must not exceed 10.
- The maximum number of “stages” within a program must not exceed 10. The following program steps or step combinations are considered stages:
 - Automatic Hot Start
 - Manual Hot Start
 - A string of up to ten single Temperature steps
 - A single Gradient step
 - A complete cycle including Temperature or Gradient steps within a Start Cycle/End Cycle pair
 - A Touchdown step
 - A Store step

To create a PCR program using the Wizard

As an alternative to manually creating a PCR program, you may use the cycler's Program Wizard. The wizard walks you through a series of five steps to automatically generate a new PCR program.

Starting the Program Wizard

- Click or touch the Program Wizard button on the Start screen.

The 5 Steps of the Program Wizard

1 Select a polymerase from the list.

- *Taq* DNA polymerase
- Paq5000 DNA polymerase (hot start or standard version)
- *PfuUltra* II DNA polymerase (hot start or standard version)
- *PfuTurbo* DNA polymerase (hot start or standard version)
- Herculase II DNA polymerase

When generating a program for Paq5000, *PfuUltra* II, *PfuTurbo*, or Herculase II DNA polymerase, the wizard algorithm determines the parameters of the program steps based on guidelines specific to that enzyme. (See the documentation for the enzyme for cycling recommendations.) These guidelines, however, are often broadly applicable and once the program is generated and displayed in the Compose a New PCR Program screen, you are able to edit the parameters of a step, or add/remove steps.

2 Enter primer information by one of two methods.

- Type in your primer sequences or browse to your sequence from a connected USB drive.
- In the absence of sequence information, enter the melting temperatures (T_m) of your primers.

3 Enter the expected product length and select the **units** as **bp** or **kb**.

4 Specify the source of template DNA.

- cDNA
- genomic DNA
- vector/plasmid DNA

5 Select adjustments to the program.

Some of the following adjustment options may not be available depending on which polymerase was previously selected.

- Hot start
- Touchdown PCR
- High GC or high AT content in the DNA template
- Gradient in block temperature during annealing

When any enzyme other than Taq polymerase is selected, the wizard will not include the Touchdown option. For these enzymes, touchdown PCR is assumed to be unneeded, so this adjustment will not be seen in the list of adjustment options in the final step of the wizard. However, once the program is generated and displayed in the Compose a New PCR Program screen, you are able to make changes.

Once the wizard has guided you through these five steps, it creates a PCR program based on the provided information and display it in the Compose a New PCR Program screen with the steps of the program displayed in order in the Program Window.

At this point, you may add, edit or delete steps in the same way as described for a manually created program.

To view or edit the parameters of any step, select the step in the Program Window and click or touch the Edit button. A dialog window will open showing the settings for that command, such as temperature and duration. See the section “[Program Steps in the Commands List](#)” on page 34 for more information on the parameters for each command.

Editing PCR Programs

Programs that are created manually or by the Program Wizard can be edited in the same way with the functions available in the Compose a New PCR Program screen. After you edit a PCR program, the program can be saved with the same name or saved under a new file name using the **Save As** button. The **Save As** feature is useful for creating new PCR programs when an existing program of a similar setup already exists.

To edit program step parameters

- 1 Click or touch **Load/Edit** on the Start screen.
Depending on your user level (Guest or registered user), the specific user directory or Guest directory is displayed listing all existing PCR program files.
- 2 Select the program to edit and click or touch **Open**.
The program steps are displayed in the Program Window panel of the Compose a New PCR Program screen.
- 3 To edit a particular step, double-click the program step, or select the step and touch the **Edit** button.
The dialog window for that program step opens.
- 4 Edit the parameters as needed and click or touch **OK**.
- 5 Repeat [step 3](#) and [step 4](#) for each program step that requires editing.
- 6 In the Compose a New PCR Program window, select **Save** to save your changes under the same program name or **Save As** to assign a new file name.
- 7 You can select **Run** at this time to start the PCR program.

To add or delete a PCR step

- Go to the Compose a New PCR Program screen for the program, then:
 - To add a new step, drag a command from the **Command List** into the Program Window in the desired location.
 - To delete a step from the program, drag the step you want to remove from the Program Window to the trash bin icon. Select **Save** to save your changes, or **Save As** to save the program under a new name.

To delete a PCR program

- 1 Click or touch **Load/Edit** in the Start screen.
Depending on your user level (*Guest* or registered user), the specific user directory or Guest directory is displayed listing all existing PCR program files.
- 2 Select the program to remove.
- 3 Click or touch **Delete** to remove the program from the folder.

Running a PCR Program

To run a PCR program

- 1 From the Start screen, click **Start**.

Your personal file folder opens or, if you are logged in as Guest, the Guest folder opens to display the available PCR programs.

- 2 Select the program to run.

- 3 Click **Run selected** to start the program.

While the program is running, a thermal profile view (a running line tracking between 0 and 100°C) is displayed on the screen next to a list of the program steps. The current step in the list will be highlighted and the step number will be displayed within the thermal profile view (for example, if the cycler was on Step 2 of the program, this step would be highlighted and a white “2” would appear at the front of the running profile line). You have the option of stopping the run, pausing the run, returning to the Start screen, or viewing program information using the buttons at the bottom of the screen. The Info screen will display current and target temperatures, elapsed time, remaining time, and other information.

To stop a program

After a run is started, the **Start PCR** button in the Start screen menu changes to **Stop**.

- Click or touch **Stop**.

To open the cycler turn the knob located on the top of the instrument. The manual abortion of the program will be recorded in the GLP and LabBooks reports.

You may also pause a run from the same screen.

Preloaded PCR Programs for Stratagene products

The Stratagene gradient cycler comes preloaded with a set of PCR programs for use with the following Stratagene PCR enzymes and site-directed mutagenesis kits. The preloaded PCR programs are in the **Guest/Agilent** folder.

- Paq5000 DNA Polymerase
- *PfuUltra* II DNA Polymerase
- *PfuTurbo* DNA Polymerase
- Herculase II DNA Polymerase
- QuikChange Lightning Site-Directed Mutagenesis Kit
- QuikChange II Site-Directed Mutagenesis Kit
- QuikChange Multi Site-Directed Mutagenesis Kit

Please note that the preloaded PCR enzyme programs are designed for amplifying targets of 1 kb or less with no special adjustments (e.g. high GC content). The parameters of the PCR steps may need to be adjusted for your particular target.

The preloaded programs for use with the QuikChange site-directed mutagenesis kits have settings appropriate for the control reactions provided with each kit. Edit the times and temperatures of the program steps as needed for your target.

To edit preloaded PCR programs

- 1 Save a copy of the program to your own user directory.

The preloaded PCR program files are read-only, so you need to make a copy before you can make edits.

- 2 Click **Load/Edit** to open the program.

- 3 In the Compose a New PCR Program screen, click or touch **Save As** to save the program file to a selected location. The newly saved file may then be edited as desired.

3 PCR Programs

To run a preloaded PCR program

To run a preloaded PCR program

- 1 Click **Start** from the Start screen.
- 2 In the directory that opens, find the Guest/Agilent folder where the preloaded programs are stored.
- 3 Select the desired program and touch or click **Run** selected to start the program.

Importing and Exporting Files

The USB drive on the front of the instrument may be used to connect to a memory stick or other external memory card, thus allowing files to be imported to and exported from the cycler. You may import primer sequences and PCR programs created on a different thermal cycler. You may also export PCR programs. For instructions on exporting report files, see [“To archive reports”](#) on page 33.

To export program files to a USB Archive

- 1 Insert the USB memory stick (or other USB storage device) into the USB slot on the front of the instrument.
- 2 Click **View & Edit Files** to display your personal file folder.
- 3 Select the PCR program to export.
- 4 Click **Save As** to open the Save Program dialog window.
- 5 Type the name of the program.
- 6 Mark the **Save to USB** check box. The file is saved to the USB drive.

To import program files to the thermal cycler

To import primer sequences for use with the Program Wizard, save each sequence in separate text files (.txt file extension). Each file should include only a string of base sequences; no titles or column headers are required.

- 1 Insert the USB memory stick (or other USB storage device) into the USB slot on the front of the instrument.
- 2 Click **View & Edit Files** to display your personal file folder.
- 3 Click or touch **View USB** to access the USB drive.
- 4 Select the file to import from the directory.
- 5 Click/touch **Save As** to open the Save Program dialog window.
- 6 Type a file name and select a path.
- 7 Click **OK** to save the file.

Reports

To view reports

- 1 On the Start screen, click or touch **Reports**.
This opens the Browse Generated Reports window listing the report files.
- 2 Group the displayed reports by selecting one of the sorting criteria below:
 - Group by Date: Sorts records in ascending order by date
 - Group by Cyclers: Sorts records by cyclers name
 - Group by Program: Sorts records by program name
- 3 Select the report to view and click or touch the **View** button.

Depending on the assigned user level, you are allowed to view and print LabBooks reports and GLP reports stored in the following file folders:

Table 5

User rights	File folder	Action
Guest users	Guest folder	Access to LabBooks reports permitted Access to GLP reports denied
Registered users	Guest folder and Personal file folder	Access to LabBooks reports permitted Access to GLP reports denied
Administrator	All guest and user folders	Access to LabBooks reports permitted Access to GLP reports permitted

To delete reports

Only users with administrator rights are authorized to delete reports.

- 1 On the Start screen, click **Reports**.
- 2 Select the report to be deleted and click or touch the **Delete** button.

To archive reports

- 1 On the Start screen, click **Reports**.
- 2 Insert a memory stick into the USB port. An Archive Reports icon appears at the top of the screen.
- 3 Select the reports to be archived and click or touch the icon to save the selected reports to the USB drive.

Program Steps in the Commands List

The following program steps are available in the Command List for creating customized PCR programs on the Compose a New PCR Program screen. The description of each step includes its specific parameters that must be defined when creating the program.

Barcode Input

If you plan to enter a barcode for your PCR plate, use this program step to define barcode settings.

Place the Barcode Input step at the beginning of the PCR program. When the program is started and the *Barcode Input* step is reached, you will be asked to read the plate's barcode. You can do this with a handheld USB barcode scanner (connected via the USB port) or by manually entering the barcode. If you use the barcode scanner, the barcode cannot be edited after reading. The program continues after you have placed the plate into the cycler and clicked OK. The barcode will be recorded in the GLP and LabBooks report and displayed in the Info section of the Cycler screen.

**Barcode Input
Before Run**

Select the Scan Using Barcode Entry radio button if you will use a barcode scanner. Select the Scan Using Keyboard Entry radio button if you will manually enter the barcode using the virtual keyboard or an external keyboard.

**Barcode Input to
read program
from worklist**

Check this box if you want to track barcodes in a work list.

Path for worklist

Specify the directory path for the work list file.

Automatic Hot Start

The *Automatic Hot Start* program step is an extended denaturation step that may be added to the beginning of PCR programs that use polymerases with hot start technology. The extended denaturation step activates the polymerase, typically by either inducing a conformational change in the enzyme or by degrading a polymerase-bound antibody.

When using the *Automatic Hot Start* program step, you specify the denaturation temperature and time. During the program run, the next command will be executed immediately after the denaturation time is elapsed.

Denaturation Temperature: Enter the temperature for the Automatic Hot Start step.

Hold for Enter the duration of the step. *Please consult the polymerase manufacturer's literature for recommendations on hot start conditions.*

Manual Hot Start

With the *Manual Hot Start* program step, you select the denaturation temperature and time. The instrument will notify you after the denaturation time is elapsed. You will then be asked to open the cycler and add the enzyme. During this time, the instrument timer is on hold and will continue only after you have pressed OK in the displayed Hot Start information window.

Denaturation Temperature Enter the temperature for the Manual Hot Start step.

Hold for Enter the duration of the step.

Heated Lid

Use this program step to set the required lid temperature. It is recommended to place the *Heated Lid* command at the beginning of the PCR program. The heated lid will only activate for steps above 25°C.

Lid Temperature Enter the temperature of the heated lid. The temperature must be between 111°C and 120°C with minimum increment of 1°C.

Lid is ON: Check this box to activate lid heating.

Temperature Step

This program step holds the thermal block at a specified temperature for a specified time. The user-defined parameters for this step also allow you to set temperature increments or decrements with each cycle and set the rate at which the thermal block reaches the set temperature.

Processing Temperature Enter the temperature for the step.

Hold for Enter the duration of the step.

Temperature Ramp Enter the desired ramp rate or choose the option to use the maximum ramp rate.

Temperature Increment If the *Temperature* step is part of a cycle, this parameter may be used to add a specified increase or decrease in the processing temperature with each cycle. Values can be from 0.1°C to 10°C. Use negative numbers (e.g. -2°C) to indicate an incremental decrease in temperature.

Time Increment: If the *Temperature* step is part of a cycle, this parameter may be used to add a specified increase in the duration of the step with each cycle. Time increments are in seconds per cycle

Step Type For informational purposes, select the type of PCR step (annealing, denaturation, or elongation) that this *Temperature* step is being used for. The step type information will be displayed in the cycler monitor.

Touchdown

Touchdown PCR is an advanced PCR technique used to reduce nonspecific primer/template binding. In the early cycles of touchdown PCR, the annealing temperature is set relatively high to promote specific amplification. Then, in later cycles, the annealing temperature is lowered to permit more robust amplification. The *Touchdown* program step is provided as a one-step command for easy programming of touchdown PCR.

Denaturation Temperature and Duration	Enter the denaturation temperature and duration of denaturation to be used during touchdown cycling.
Maximum Annealing Temperature	Enter the maximum annealing temperature.
Minimum Annealing Temperature	Enter the minimum annealing temperature.
Duration (for annealing)	Enter the duration of the annealing step.

NOTE

The difference between the maximum and minimum annealing temperatures is commonly between 5°C and 10°C starting with a maximum temperature that is 2°C above the higher primer melting temperature (T_m). For example, if the T_m of Primer 1 is 60°C and the T_m of Primer 2 is 54°C, then the maximum temperature should be 62°C and the minimum temperature should be around 52°C.

Elongation Temperature and Duration	Enter the temperature for elongation and the duration.
Cycles	Enter the number of cycles for the <i>Touchdown</i> segment.

Gradient Step

In this program step, a temperature gradient is generated across the thermal block for a specified length of time. The *Gradient* step is useful for optimizing the annealing temperature for your particular primer/template system.

Minimum Temperature Gradient Enter the minimum temperature of the gradient. The value must not be below 30°C.

Maximum Temperature Gradient Enter the maximum temperature of the gradient. The value must not exceed 99°C.

The difference between the minimum and maximum temperatures must be between 4°C and 30°C.

Hold for Enter the duration of the *Gradient* step

Convert Gradient Step to a standard temperature step Once the optimal annealing temperature has been determined, the *Gradient* step can be easily converted to a standard *Temperature* step by marking this check box and specifying which temperature column yielded the best amplification results. The converted *Gradient* step will then automatically use the temperature of this best-performing column. To convert back to a *Gradient* step, clear the Conversion check box.

Start Cycle

This program step starts a PCR cycle. Put this step before a series of steps that are to be repeated for several cycles.

Cycle Name Enter a user-selected name for the cycle.

Number of Loops Enter the number of times the series of steps should be repeated. The maximum is 99.

End Cycle

Insert this program step to terminate a PCR cycle. Put this step after a series of steps that are to be repeated for several cycles.

NOTE

To have a valid program, the steps of a “loop” need to be contained between a Start Cycle and an End Cycle command.

Store

The *Store* program step cools the thermal block down to a temperature between 4° and 12°C for a specified period of time or indefinitely. During the *Store* step, the lid pressure is set to zero Newton.

Store Temperature Enter the storage temperature in °C.

Hold for Select this radio button if you prefer to maintain the store temperature for a set duration of time. Enter the duration in the accompanying field.

Infinite Select this radio button to hold the specified store temperature for an unlimited period of time.

Program Information

Use this program step to record any comments about the PCR program.

3 PCR Programs

Program Information



4 System Management

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This chapter contains instructions to set up user accounts and cycler settings.



User Accounts

An administrator may use the Settings menu (accessible from Start screen) to set up new users, edit user account information and delete user accounts.

To add new user accounts

- 1 To add a new user, click **Settings** in the Start screen.
- 2 Select **User Management** from the menu.
The User Management screen opens listing the available user names and corresponding user levels.
- 3 Select **New User** to open the Add a New User screen.
- 4 Assign a user name and password.
- 5 Select the user level (**User** or **Administrator**) from the drop-down menu.
Selecting **User** will create a registered user account. Selecting **Administrator** will give the user administrator rights.
- 6 Mark the appropriate check boxes to define the user's read and write privileges.
 - **Edit/Rename Script:** The user has the right to edit or rename a program file.
 - **Delete Script:** The user has the right to delete program files.
 - **Create Script:** The user has the right to create program files.
- 7 Click or touch **OK** to save the settings.

After successful setup, a confirmation message appears and the new user name is added to the User Management list.

To edit user accounts

- 1 To change an existing user account, select **Settings** from the Start screen.
- 2 Select **User Management** from the menu.
The User Management screen appears listing the available user names and corresponding user levels.
- 3 Select the user account to edit and click or touch **Edit User**.
- 4 From the Edit a User screen, change data such as name, user level, password, user privileges and program directory protection.
- 5 Click or touch **OK** to save the changes.
A confirmation message appears after successful update of the user data.

To delete user accounts

The Administrator account cannot be deleted.

- 1 Select **Settings** from the Start screen.
- 2 Select **User Management** from the menu.
The User Management screen appears listing the available user names and corresponding user levels.
- 3 Select the user name to remove.
- 4 Click or touch **Remove User** to remove the user name from the user list.
All user permissions for that account are deleted. All programs and GLP reports associated with that user are deleted.

Cycler Settings

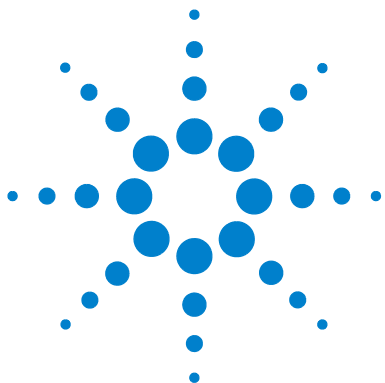
To edit cycler data

The name and details of a connected unit can be changed by an administrator using the following steps:

- 1 From the Start screen, click or touch **Settings**.
- 2 Select **Cycler Management** from the menu.
- 3 Click or touch the name of thermal cycler and click **Edit Cycler Settings**.
- 4 Make the desired changes in the Cycler Settings screen.
- 5 Confirm your changes by clicking or touching **OK**.

To set the time and date

- 1 Click or touch **Settings** from the Start screen.
- 2 Select **Cycler Settings** from the menu.
- 3 To change the date:
 - a Select the month, date or year in the System Date field.
 - b Click or touch the up and down arrows to adjust.
- 4 To change the time:
 - a Select the hour, minutes or seconds in the System Time field.
 - b Click or touch the up and down arrows to adjust.
- 5 Confirm your changes by clicking or touching **OK**.



5 Installation and Maintenance

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This chapter contains installation and maintenance instructions.



Installation

Step 1. Select a location for the instrument

- Locate a solid, flat clean surface for the instrument. Make sure:
 - The instrument can stand completely stable.
 - The rear and side air slots will not be covered and air can circulate freely around the instrument. Adequate ventilation is important.
 - The unit always has at least 10 cm (approximately 4 inches) to the next wall or neighboring instrument.
 - No paper sits under the instrument as this may block the ventilation path.
 - The temperature (normal ambient) is between 10°C and 30°C with humidity levels between 0% and 95%.
 - The atmosphere is not explosive.

Step 2. Unpack the cycler

- 1** Open the Stratagene gradient cycler shipping container and remove the three boxes inside.
The largest of the boxes contains the instrument.
- 2** Remove the instrument from its box and place in the selected location.
- 3** Unpack the contents of the smaller two boxes.
One contains the power cord and the other contains the thermal block.

Step 3. Insert the thermal block

CAUTION

Make sure power is off and thermal cycler is disconnected.

- 1 Lift the lid to expose the recessed area where the block will sit.
- 2 Hold the block with two hands on the left and right side, with the block connector at the back of the block.
- 3 Place the thermal block into position in the cycler. The block will snap into place.

Step 4. Connect the instrument to a power supply

The thermal cycler must be connected to a grounded AC outlet.

- 1 Plug the thermal cycler's power cord into the power connector at the rear of the instrument.
- 2 Connect the other side of the cable to the outlet.

Step 5. Connect optional devices

You can connect optional input devices such as a keyboard, mouse, and barcode reader to the thermal cycler systems via a USB connection.

- 1 Plug a USB cable (not provided) into the USB port of the input device.
- 2 Connect the other end of the USB cable into the USB port on the thermal cycler.

If you plan to connect multiple USB devices into the instrument, you can use an external USB hub.

Step 6. Turn on the instrument

- Press the power button on the front of the instrument.

You can turn off the instrument any time. You do not need to close the thermal cycler software before you turn off the instrument.

To change the thermal block

CAUTION

Turn power off and disconnect power supply before you change the block.

The thermal blocks available for the Stratagene gradient cycler are interchangeable.

- 1 Remove the current block:
 - a Hold the installed block with two hands on the left and right side
 - b Lift the block straight up.
- 2 Insert the new block:
 - a Hold the block with two hands on the left and right side, with the block connector at the back of the block.
 - b Place the block into position in the thermal cycler. The block will snap into place.

Maintenance

Cleaning

The Stratagene gradient cyler is designed to require a minimum amount of maintenance by the user.

- Use water or a mild laboratory-cleaning agent to clean the instrument.
- Do not let organic solvents or aggressive solutions come in contact with the instrument.
- Do not let liquid enter the thermal cyler.
- Turn off and disconnect the instrument from the power supply before you clean.
- Do not service the thermal cyler unless you are qualified to do so.

Replacing a fuse

WARNING

Disconnect the power cord before you remove or install a fuse to avoid the possibility of serious injury from electrical shock.

Fuse compartments are located on the rear of the instrument, above the main power connection. Check the voltage rating label to verify that the instrument is compatible with the AC voltage available at the installation site. Check that fuses are rated as T 6.3A, 250V. Purchase replacement fuse sets from Agilent (part number G5100-80001, 2 fuses per set).

Troubleshooting

This section describes potential problems and recommended actions.

If no actual temperature is displayed

The block may not be correctly inserted in the instrument.

- ✓ Check that the block is inserted correctly and reboot the cycler.

If the program does not start

The block may not be correctly inserted in the instrument.

- ✓ Check that the block is inserted correctly and reboot the cycler.

If tubes are crushed

- ✓ Lid pressure is too high. Reduce pressure using lid knob.

If condensation is found in tubes during a run

Condensation will appear naturally at the end of a run and has no deleterious effects.

- ✓ Spin the liquid back to the bottom of the tube and continue with the post PCR steps. However, condensation during a run will result in sub-optimal cycling. If this occurs, increase the lid temperature.

If the sealed microplate loses sample volume

- ✓ Check that the microplate/sealing system is of good quality.
- ✓ Try to increase the pressure on the lid by turning the knob.

If USB devices including memory stick and barcode scanner not recognized

- ✓ Reboot the cycler.

If cycler does not turn on

- ✓ Check that the power is on at supply.
- ✓ Check fuses at rear of instrument above power inlet.

If Blank Display is lit but shows no information

- ✓ Reboot the cycler.
- ✓ If the problem persists there is an internal connection problem; call Technical Support. See [“Technical Support”](#) on page 2.

If the power fails

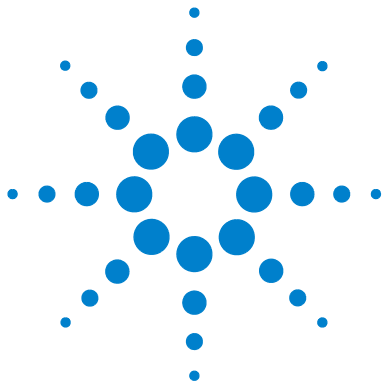
The thermal cycler is equipped with a power failure restart function that, in the event of a power failure, allows the instrument to automatically resume a PCR run after power is returned:

- The PCR program restarts from the beginning of the temperature step in which it failed.
- The Cycler Settings screen (accessible from the Systems Settings screen) has a selection area for enabling and disabling the *Power Resume* function.

The following table lists three power failure scenarios and the actions the instrument takes if the power is interrupted during operation.

Table 6 Power Failure Responses

Failure	Instrument Response
The computer fails but the cycler unit still has power	The thermal engine will continue to execute the program without any interruption.
The cycler fails but the computer still has power	The execution of the program will halt while the power is off. Once power returns, restart the unit and the program will resume from the beginning of the step it was executing when the power failed.
Both the computer and the cycler unit lose power	The execution of the program will halt while the power is off. Once the power returns, the program will resume from the beginning of the step it was executing when the power failed.



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This chapter contains reference information for using your thermal cycler.



Compatible Plasticware Formats

The following PCR plates and tubes are compatible with the cycler:

Agilent	Mx3000P/Mx3005P 96-well tube plates, 410088 Mx3000P/Mx3005P strip tubes, 401428 Mx3000P/Mx3005P optical strip caps, 401425
ABgene	AB 800 96-well, skirted AB 900, 96-well, semi-skirted AB 1111, 384-well* TF-0384, 384-well*
Biatec	THERMOSPRINT 96 PP, transparent, 311296 THERMOSPRINT 384 PP, transparent, 310384*
NUNC	Art. No.: 240600, 96 well, non-skirted. Art. No.: 230013, 96 well, semi-skirted Art. No.: 230012, 96 well, skirted Art. No.: 230584, 384 well*
Eppendorf	Art. No.: 0030 128.648, twin tec PCR plate, 96 skirted Art. No.: 0030 128.575, twin tec PCR plate, 96 semi-skirted Art. No.: 0030 128.508, twin tec PCR plate, 384*
Corning	Thermowell 96 Well Polypropylene PCR Microplate, Natural, #6551 Thermowell 96 Well Polycarbonate PCR Microplate, Model M, #6511 Corning 384 Well Polypropylene PCR Microplate, #6500* Corning 384 Well Polyethylene PCR Microplate, #6502*

*For best results with the 384-well block, polypropylene plates are recommended.

Related Agilent Reagents

Description	Part Number
<i>PfuUltra</i> II Fusion HS DNA Polymerase, 40 Rxn	600670
<i>PfuUltra</i> II Fusion HS DNA Polymerase, 200 Rxn	600672
<i>PfuUltra</i> II Fusion HS DNA Polymerase, 400 Rxn	600674
Herculase II Fusion DNA Polymerase, 40 Rxn	600675
Herculase II Fusion DNA Polymerase, 200 Rxn	600677
Herculase II Fusion DNA Polymerase, 400 Rxn	600679
Paq5000 DNA Polymerase, 500 U	600680
Paq5000 DNA Polymerase, 1000 U	600682
Paq5000 DNA Polymerase, 5000 U	600684
Paq5000 Hotstart DNA Polymerase, 1000 U	600860
Paq5000 Hotstart DNA Polymerase, 500 U	600862
Paq5000 Hotstart DNA Polymerase, 5000 U	600864
<i>Taq2000</i> DNA Polymerase, 100U	600195
<i>Taq2000</i> DNA Polymerase, 500U	600196
<i>Taq2000</i> DNA Polymerase, 1000U	600197
SureStart Taq DNA Polymerase, 100U	600280
SureStart Taq DNA Polymerase, 500U	600282
SureStart Taq DNA Polymerase, 1000U	600284
Deoxynucleotide Mix, PCR Grade	200415
QuikChange Lightning Site-Directed Mutagenesis Kit, 10 rxn	210518
QuikChange Lightning Site-Directed Mutagenesis Kit, 30 rxn	210519
QuikChange II Site Directed Mutagenesis Kit, 10 rxn	200523
QuikChange II Site Directed Mutagenesis Kit, 30 rxn	200524

Instrument Specifications

Thermal Blocks

Block Materials	Modular anodized aluminium or gold coated silver blocks with 4 sensors
Traceability	NIST traceable temperature calibration
Blocks Available	96-well thermal block 96-well fast-cycling thermal block 384-well high-throughput thermal block

High Pressure Heated Lid

Lid Temperature Range	111°C to 120°C, user selectable in 1°C increments
Lid Pressure Range	Pressure set manually

Power & Dimensions

Electronic Power Supply	90V to 250V (frequency 48 to 62Hz)
Dimensions	(W×D×H) 34 cm × 42 cm × 26 cm
Weight	Approximately 26 pounds
Operating conditions	10°C to 30°C, 0 to 95% relative humidity
Regulatory	CE, UL, CSA compliant
Warranty	2 years on all systems

Thermal Engine Characteristics

Temperature Control	4°C to 99.9°C with simulated volume dependent control
Sample Volume Range	5 to 100µL
Sample Temperature Accuracy	+/-0.1°C
Sample Temperature Homogeneity	+/-0.4°C
Heating	Up to 5°C/second for 96-well fast block Up to 3°C/second for 96-well block Up to 5°C/second for 384-well block
Cooling	Up to 3.5°C/second for 96-well fast block Up to 2°C/second for 96-well block Up to 3.5°C/second for 384-well block
Sample Overshoot	<1°C
Gradient Temperature Range	30°C to 99°C
Maximum/Minimum Gradient Span	30°C/4°C

Compatible Barcodes

The following barcodes are compatible with the Stratagene gradient cycler documentation system:

- 2 of 5 interleaved
- Codabar
- Code 128
- Code 39
- Code 39 full ASCII
- Code 93 full ASCII
- EAN-13, EAN -13 with 2 supplement, EAN -13 with 5 supplement
- EAN-8, EAN -8 with 2 supplement, EAN -8 with 5 supplement
- EAN-128
- UPA, UPA -13 with 2 supplement, UPA -13 with 5 supplement
- UPE, UPE -13 with 2 supplement, UPE -13 with 5 supplement
- MSI
- C.I.P

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In This Book

This document describes how to program and use the Stratagene Gradient Cycler.

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