

Standard Operating Procedure

SOP191

Title: Use and Maintenance of the Applied Biosystems 96 well fast thermal cycler

Location: CBE (H34)

1. PURPOSE

The intent of this SOP is to describe the use and maintenance of the Applied Biosystems 96 well fast thermal cycler (Veriti) 0.2mL.

2. SCOPE

This SOP applies to the Applied Biosystems Veriti 96 well thermal cycler in H34.

This SOP assumes that the user knows how to handle DNA and/or RNA samples and is able to prepare them for PCR.

Tutorials, Guides and Troubleshooting documents for first time and other users are available at <http://www3.appliedbiosystems.com/AB Home/Support/TutorialsTroubleshooting/index.htm#>

This SOP does not cover the experimental procedures that can be run on the instrument. These should be derived on a case-by-case basis using the guidelines signposted in this SOP. Protocols for the experimental types described in this document should be derived to support the risk assessment of the individual work activity e.g. under COSHH assessment of reagent kits.

SPECIAL NOTES: HEALTH & SAFETY

(i) General Instrument Safety

Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

- The instrument must be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide (see user manual) If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

(ii) Chemical Safety

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Wastes produced by this PCR instrument are potentially hazardous and can cause injury, illness, or death. Refer to local SOP039 for chemical storage, handling, and disposal procedures.

Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.

The MSDS for any chemical supplied by Applied Biosystems is available from <http://www3.appliedbiosystems.com/sup/msds/search.htm>.

NOTE: Each time you receive a new MSDS packaged with a hazardous chemical, ensure that the appropriate MSDS is replaced in the Master files.

- Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, lab coat, and gloves when handling reagent and waste bottles.
- Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, and lab coat).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS. Refer to SOP039 for further guidance - record spill in the Spill Record Log.
- **Avoid the use of mutagenic gel staining reagent. The use of Ethidium Bromide is not permitted in the CBE Laboratories. Working concentrations of GelRed for example, which is a non-mutagenic and non-cytotoxic dye can be used for gel staining.**

(iii) Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in the Laboratory Unit.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local SOP, COP and national regulations.
- **CAUTION:** Mixed chemical and biohazardous waste materials may require special handling, and disposal limitations may apply. Consult the relevant MSDS. Refer to SOP039 and SOP003 for guidance.

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(iv) Electrical Safety

- Severe electrical shock can result from operating the StepOne instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.
- Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in the laboratory unit.
- For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.
- Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.
- Use properly configured and approved line cords for the voltage supply in your facility. ➤ Plug the instrument into a properly grounded receptacle with adequate current capacity.

(v) LED Safety

To ensure safe LED operation:

- The system must be maintained by an Applied Biosystems Technical Representative.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the LED is operating (during service with safety interlocks disabled), you may be exposed to LED emissions in excess of the Class 3B rating.
- Do not remove safety labels or disable safety interlocks.

(vi) Biological Hazard Safety

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local SOP, COP and/or national regulations.

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- Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves.
- All work with viable biological materials should be performed in using physical containment devices such as a BSC. NOTE: Once the biological material has been added to the lysis buffer employed in the extraction procedure and rendered non-viable, subsequent extraction of the genomic sample can be carried out outside the BSC if necessary.



(vii) Workstation Safety

Correct ergonomic configuration of the workstation can reduce or prevent effects such as fatigue, pain, and strain.

- Minimize or eliminate these effects by configuring the workstation to promote neutral or relaxed working positions.
- To minimize musculoskeletal and repetitive motion risks:
 - Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
 - Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

3. RESPONSIBILITIES

CBE Laboratory Users

- They have read and understood all applicable Material Safety Data Sheets (MSDSs).
- They have an approved COSHH assessment for the experimental run supported by a written protocol.

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- Use the equipment as instructed and report any issues to Laboratory Manager.

Responsible Person (RP)/Laboratory Manager (LM)

- All users of the instrument have received instructions in both general safety practices and specific safety practices for the instrument.
- All users have read and understood all applicable Material Safety Data Sheets (MSDSs).
- All Users have been trained and authorised to operate the instrument.
- The instrument is regularly maintained.

4. EQUIPMENT AND MATERIALS

96-Well Plates

- MicroAmp™ Optical 96-Well Reaction Plate (0.2 mL) #N8010560 & #4316813
- MicroAmp™ Optical 96-Well Reaction Plate with Barcode (0.2 mL) #4306737 & #4326659
- MicroAmp™ Optical 96-Well Reaction Plate with Barcode and Optical Caps (0.2mL) #403012
- MicroAmp™ Optical 96-Well Reaction Plate with Barcode and Optical Adhesive Films (0.2mL) #4314320

12-well strips

MicroAmp™ 12-Cap Strip (0.2mL) #N8010534 & N8011534


5. PROCEDURE

Using the touchscreen

You interact with the Veriti™ Thermal Cycler using a touchscreen.

Table 1 describes several buttons common to many of the touchscreens.

Table 1 **Buttons in the Veriti™ thermal cycler touchscreen**

Button	Function
	Returns to the Main Menu screen.

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	Returns to the previous screen.
	When a run is in progress, displays the Status Report to view any errors that occurred during the run.
	When a run is in progress, displays the Run Monitor screen to view the run.
	Scrolls up and down through a list of items.
	Displays the run method folders.
	Creates a “shortcut” to the selected run method (or methods).
	Indicates that a USB drive is connected to the instrument’s USB port.
	Opens the Help system.

When you touch a field that requires the input of text, the text editor opens (Figure 2).



Figure 2 The Veriti™ Thermal Cycler text editor

Using the touchscreen

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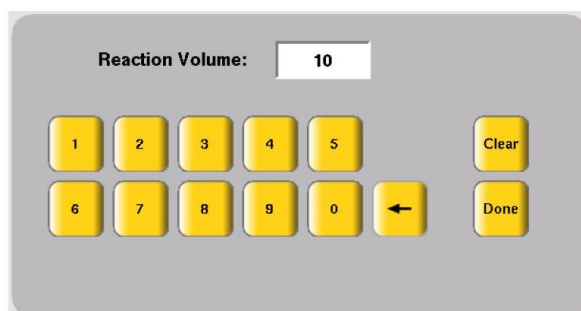
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To...	Select...
Enter a lowercase letter	A letter button.
Enter an uppercase letter	 Caps , then the letter(s). When you are done, select Caps again.
Enter punctuation or other symbols	 Symbols then the symbol(s). When you are done, select Symbols again.
Remove the contents of the entire field	
Delete the last letter	
Close the editor and save any changes	

Entering When you touch a field that requires only numerical input, the numeric editor opens numerals (Figure 3).



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Figure 3 The Veriti™ Thermal Cycler numeric editor

To...	Select...
Enter a number	A number button.
Delete the last number	
Remove the entire contents of the field	
Close the editor and save any changes	

Menu overview

Menu overview

Main menu screen

After you log in to the system, the Main Menu screen opens. This screen is the starting point for operating the Veriti™ Thermal Cycler. The date, current time, and current user are shown at the bottom of the screen.

If your system does not require login, the My Account button is not shown.

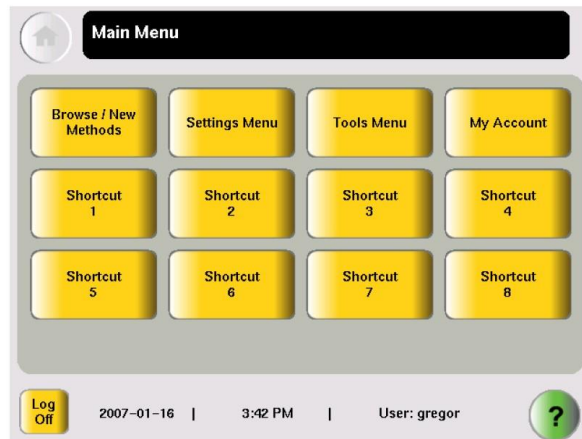


Figure 4 The Veriti™ thermal cycler main menu screen

In the Main Menu screen, select:

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- **Browse/New Methods** to view and work with all the run methods to which you have access (see “Viewing a run method“ on page 14).
- **Settings Menu** to access various instrument configuration options. If you have administrative privileges, you access the Admin Menu screen from this screen (see “Settings menu screen“ on page 12).
- **Tools Menu** to access instrument tests, tools such as a Tm calculator and run method conversion, as well as viewing the results of the last run and statistics about the instrument (see “Tools menu screen“ on page 13).
- **My Account** to view and edit your account information. If your system does not require login, this button is not shown.
- The **Shortcut** buttons allow you to configure instrument functions you frequently use to appear in the Main Menu screen (see “Configuring main menu shortcuts to functions“ on page 35).
- **Log Off** to log off the instrument. You can still log off when a run is in process. If your system does not require login, the  (Power) button is shown instead.
- , (Help) to access the Help system.

Tools menu Use this screen to access various tools and instrument tests. screen

About run methods

What is a run method? A run method is a set of instructions that specifies how the instrument should heat and cool your samples. Run methods are stored in the instrument firmware.

Predefined methods

The Veriti™ Thermal Cycler firmware has 10 pre-defined run methods.

- AmpliCycle™ Seq Run Method
- AmpliTaq Gold™ DNA Polymerase Run Method
- AmpliTaq Gold™ Fast PCR Run Method
- BigDye™ Kit Standard Run Method
- BigDye™ Kit Fast Run Method
- Fast PCR Run Method
- General PCR Run Method
- LMS2 Run Method
- Time Release Run Method
- Touchdown PCR Run Method

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- XL PCR Run Method


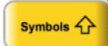
Each method is stored in the Shared folder. You can edit, copy, and run these predefined methods as you would any other run method, but you cannot delete them.

When you touch a field that requires the input of text, the text editor opens (Figure 2).



Figure 2 The Veriti™ Thermal Cycler text editor

Using the touchscreen

To...	Select...
Enter a lowercase letter	A letter button.
Enter an uppercase letter	 Caps , then the letter(s). When you are done, select Caps again.
Enter punctuation or other symbols	 Symbols then the symbol(s). When you are done, select Symbols again.

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Remove the contents of the entire field	
Delete the last letter	
Close the editor and save any changes	

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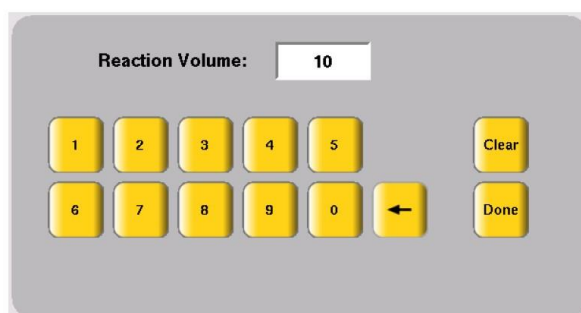





Figure 3 The Veriti™ Thermal Cycler numeric editor

To...	Select...
Enter a number	A number button.
Delete the last number	
Remove the entire contents of the field	
Close the editor and save any changes	

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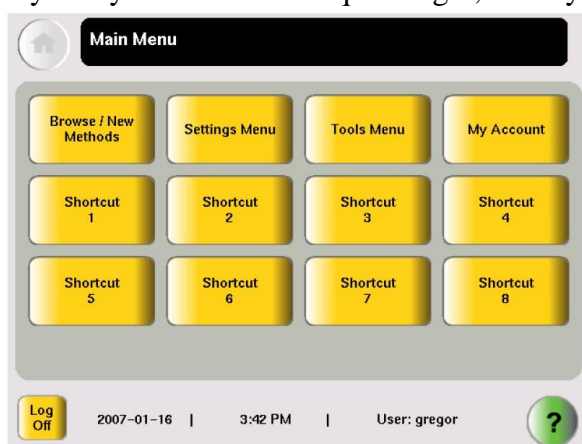




Figure 4 The Veriti™ thermal cycler main menu screen

In the Main Menu screen, select:

- **Browse/New Methods** to view and work with all the run methods to which you have access (see “Viewing a run method“ on page 14).
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Tools menu Use this screen to access various tools and instrument tests. screen

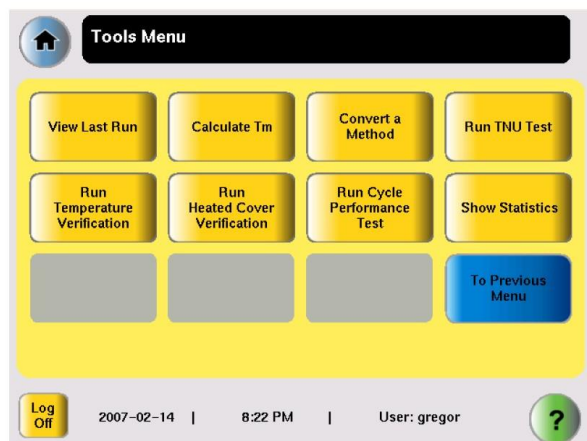


Figure 7 The tools menu screen, available to all users

For more information about the functions on the Tools Menu.

About run methods

What is a run method?

A run method is a set of instructions that specifies how the instrument should heat and cool your samples. Run methods are stored in the instrument firmware.

Predefined methods

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- o AmpliTaq Gold™ Fast PCR Run Method
- o BigDye™ Kit Standard Run Method
- o BigDye™ Kit Fast Run Method
- o Fast PCR Run Method
- o General PCR Run Method
- o LMS2 Run Method
- o Time Release Run Method

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- o Touchdown PCR Run Method
- o XL PCR Run Method

Each method is stored in the Shared folder. You can edit, copy, and run these predefined methods as you would any other run method, but you cannot delete them.

Loading samples into the instrument

- Whether using tubes, 8-well strips or 96-well plates, pipette the samples and PCR mix into the sample wells. If using tubes or 8-well strips make sure they are capped firmly. If using a 96-well plate seal using either 8-cap strips or Clear Adhesive Film.

For a 96-Well plate

- Carefully lift the plate after it has been sealed and spun down in the centrifuge, and place in the sample block.
- Place the plate onto the sample block so that the A1 well is at the upper left corner of the tray.
- Make sure that the plate is pressed down firmly and that it is properly sealed.
- Close the heated cover.

For tubes and 8-well strips.

- As above, except to place the GeneAmp Thermal isolation frame around the sample block(s)
- Close the heated lid.

Creating a new run method

1. In the Main Menu screen, select **Browse/New Methods**.
2. In the Browse Run Methods screen, select **New**. The default run method is displayed in the Edit Run Method screen.

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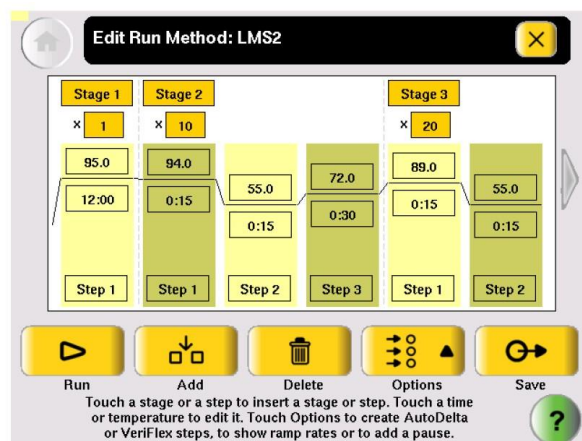
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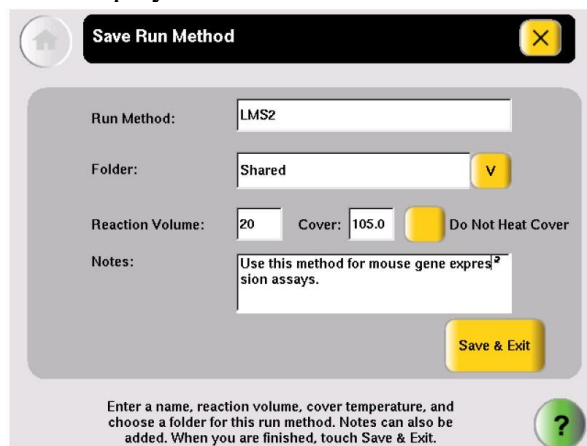
Location: CBE (H34)



3. Edit the default run method as needed:

- Insert a stage
- Insert a step
- Add an AutoDelta step
- Add a pause
- Delete a step or stage

4. Select **Save & Exit** to display the Save Run Method screen.




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5. Select **Run Method** to enter the name of the run method. Names can be up to 48 characters. Names may not contain any of the following characters: %*?|;,:!@#\$.() <>^'""~{ }[]+=&^ (tab). Spaces, underscores, and dashes are allowed.
6. Select  (Folder) to choose a folder in which to save the run method.
 - To save the run method so that all users have access to it, select **Shared**.
 - To create a new folder for the run method, select **New Folder** at the bottom of the list, enter a name of up to 12 characters (no spaces), for the new folder, then select **Done**.
 - To save the run method to the USB drive, select **USB** (available only when a USB drive is inserted in the instrument).
7. Select **Reaction Volume** to enter the reaction volume. (You can change this volume when you perform a run with this run method.) 8. Set the cover temperature, if any:
 - **Cover Temperature** to enter the temperature for the heated cover. *or*
 - **Do Not Heat Cover**.
8. You can change this temperature when you perform a run with this run method.
9. Select **Notes**, then enter any notes for the method.
10. Select **Save & Exit** when you are done. The Browse Run Methods screen is displayed.

Editing details

Adding a stage The two types of run method stages are:

- **Cycling** – The steps in a cycling stage are repeated a specified number of times. In general, the steps in a cycling stage are template denaturation, primer annealing, and primer extension.
- **Holding** – The steps in a holding stage are not repeated.

To add a stage:

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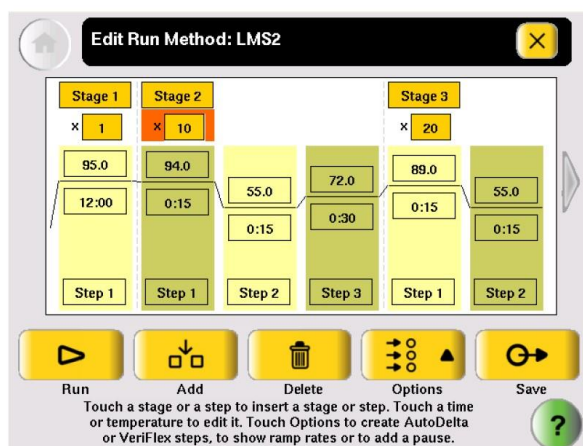
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- In the Edit Run Method screen, select the stage name (at the top of the stage) to select it. When a stage is selected, the area around the name is red.



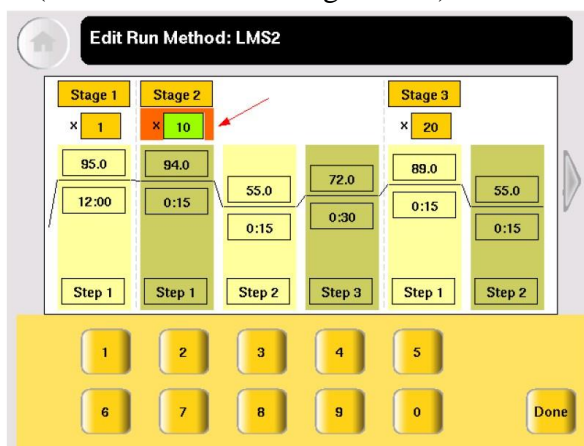
- Select **Add**.

A copy of the selected stage is added to the run method.

11. For a cycling stage, edit the number of cycles as needed.

a. Select the stage to select it.

b. Select the cycles field (the box below the Stage Name) to select it:



c. Enter the number of cycles, then select **Done**.

3. To change the temperature and/or time for the step

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- Select any temperature field, then edit the temperature as desired or select any time field, then edit the time as desired.
- You can edit any other times, temperatures, (or the number of cycles for a stage) without closing the editor.
 - Select the field.
 - Select the keys in the editor.
 - Repeat for any other field.
- Select Done to close the editor

Editing Ramp Rates

You can edit the rate at which the instrument ramps to the temperature for the step. The default ramp rate is 100%.

Note: You cannot edit ramp rates for methods created using the Convert Method wizard.

1. Select a step to select it.
2. Select **Options**, then **Ramp Rate**. The field displaying time changes to display the ramp rate.
3. Select the ramp field, then enter the desired ramp rate. Ramp rates from 0.1 to 100% are valid.
4. Select **Done** to close the editor. The ramp rate symbol **R** appears next to any step with a ramp rate less than 100%.

Creating an AutoDelta Step- An AutoDelta step allows you to increase or decrease the temperature and/or change the duration of the cycle during a cycling stage.

See the user manual for how to change this.

Adding a Pause

You can add a pause before any step in any cycling stage.

1. In the Edit Run Method screen, select step in a cycling stage to select it, then select **Options**.

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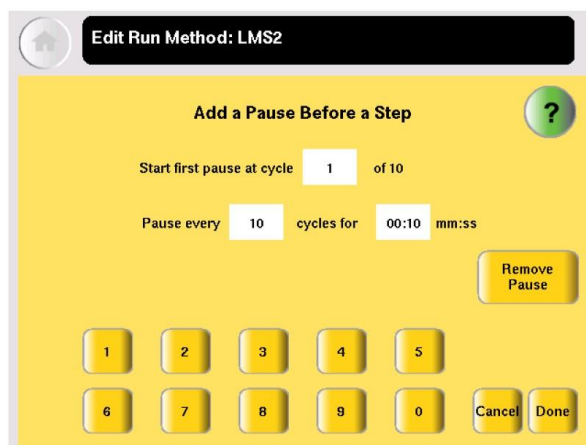
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- In the Options Menu, select **Pause** to display the Add a Pause Before a Step dialog box:

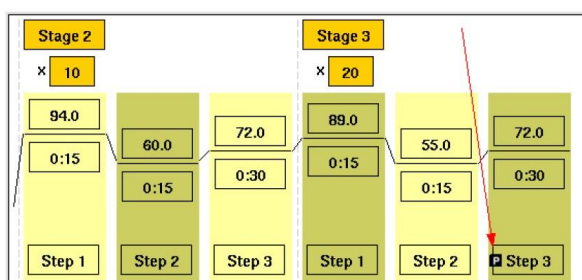


- Enter:

- The first cycle in which the run will pause.
- The frequency of the pause.
- The duration of the pause, in mm:ss.

- Select **Done** to save the pause and return to editing the method.

P



The step preceded by the pause is indicated by the (see Step 3 in Stage 3):

Deleting a stage or a step

- In the Edit Run Method screen, select the stage or step to select it.

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2. Select Delete, then OK. The stage or step is deleted from the run method.

Performing a Run.

Before you begin.

Prepare your samples and load the sample block.

To start a run from the Main Menu screen:

1. Close the cover.
2. Select **Browse/New Methods**. Locate the run method you want to use then select the run method to select it.
3. Select **Start Run**. The Run Parameters screen is displayed:

The screenshot shows the 'Run Parameters' screen with the following fields and controls:

- Reaction Volume:** 20 uL
- Cover Temperature:** 105.0 °C. There is a checked checkbox for 'Do Not Heat Cover' and a 'Current: 25.0°C' indicator.
- Run ID:** 200711130343
- Notes:** An empty text area.
- Buttons:** 'Start Run Now' and 'Start Multiple Runs'.
- Footer:** 'Touch each field and use the keyboard to edit the contents. When you are finished, touch Start Run Now or Start Multiple Runs.' and a help icon.

4. Select **Reaction Volume** to edit the reaction volume. By default, the reaction volume you entered when you saved the method is displayed.

Reaction volumes are specific to the type of instrument:

- Veriti™ 96-Well *Fast* Thermal Cycler: 5 to 40 µL
- Veriti™ 96-Well Thermal Cycler: 10 to 100 µL

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Reviewed by:

Approved by: C.Kavanagh

Standard Operating Procedure

SOP191

Title: Use and Maintenance of the Applied Biosystems 96 well fast thermal cycler

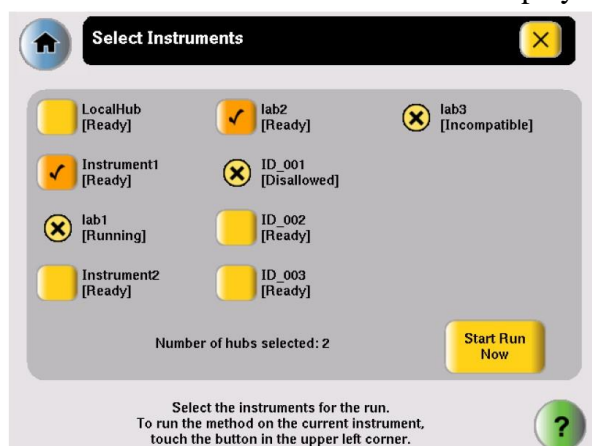
Location: CBE (H34)

- **Cover Temperature** to enter the temperature for the heated cover. *or*
- **Do Not Heat Cover.**

5. Select **Run ID**, and/or **Notes**, then enter the applicable information.

The default Run ID is YYYYMMDDHHMM, where “YYYYMMDD” is the date and “HHMM” is the current time. 8. If you are making runs on:

- **Only this instrument** – Select **Start Run Now**, then **OK**. The cover is heated to the required temperature, then the run begins. When the run is complete, the Run Report is displayed. *or*
- **Multiple instruments** – Select **Start Multiple Runs**. The Select Instrument screen is displayed:



6. In the Select Instrument screen:

- a. Select the check box corresponding to each instrument on which you want to perform the run. Only instruments that are compatible with the run method are available. Incompatible instruments are indicated by

If you want to perform the run on this instrument, be sure to select the check box in the upper left corner of the screen.

- b. Select **Start Run Now**, then **OK**. The run method is sent to each selected instrument, and, if no problems are encountered, the run begins.

After the run begins, you must interact with any remote instruments directly if you want to make changes to the run method, pause, or stop the run. When the runs are complete, the Run Reports are displayed on each instrument.

7. While the cover heats, the Cover Heating dialog box appears in the Run Monitor screen:

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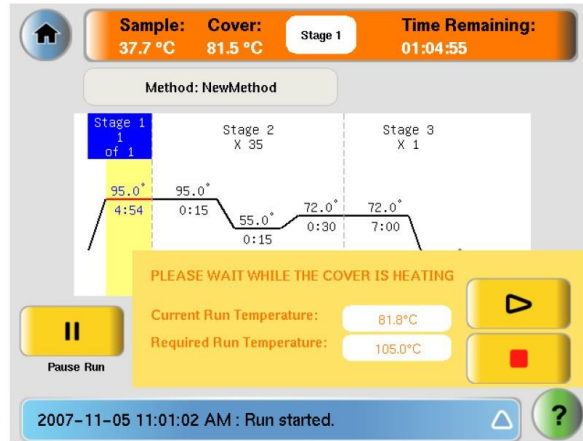
Written by: J.Bowdrey	Reviewed by:	Approved by: C.Kavanagh
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Standard Operating Procedure

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Location: CBE (H34)



If you want to:

- Start the run immediately, before the cover reaches the specified temperature – Select (Skip).
- Stop the run – Select (Stop).

Monitoring a run

Run Monitor You can follow the status of a run using the Run Monitor screen (below):

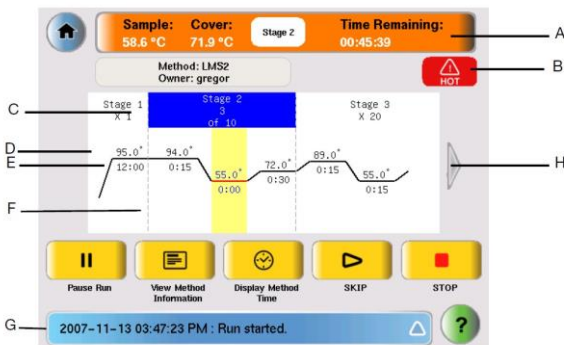


Table 5 Elements of the run monitor screen

Location in Figure	Element	Description

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Standard Operating Procedure

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Title: Use and Maintenance of the Applied Biosystems 96 well fast thermal cycler

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A	Run Monitor Button and Status Bar	Displays the estimated temperature of the sample, the temperature of the heated cover, the estimated time remaining in the run, and the current stage.
B	Hot Icon	Indicates that the heated cover has achieved a temperature sufficient to burn skin.
C	Stage Indicator	Indicates the stage that the Veriti™ System is performing and the current cycle in that stage.
D	Step Temperature	Indicates the temperature for the step.
E	Hold Time	Indicates the time for the step. When this is the current step, this field counts down.
F	Step Indicator	Indicates the step that the Veriti™ System is performing.
G	Status Report	Displays the run status and any errors that occur during the run. Select the triangle to view the entire report.
H	Arrow Button	Select this to see the additional steps in the run method.

During a run, the touchscreen is active, allowing you to perform the actions indicated in the table.

Table 6 Actions in the Run Monitor screen allowed during a run

To...	Do This
View other stages	 Use the scroll right and scroll left buttons.
Pause the run	 Select Pause Run (see page 55).
View run method information	 Select View Method Information . The information you entered in the Run Parameters dialog box is shown.

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Display the time for the run	 Select Display Method Time . The elapsed time and time remaining for the run are shown.
Skip to the next stage	
Stop the run	 Select STOP
Change the number of cycles for a cycling stage Go to the Main Menu screen	 Select (Home). From the Main Menu Screen, you can access most of the Veriti™ Thermal Cycler functions while the run continues.
View the Status Report	Select  (Status).

Changing the number of cycles

1. If needed, click the orange run monitor button to return to the run monitor screen.
2. Select the cycles field (below the Stage Name) for a cycling stage, then enter the new value. You cannot:
 - Edit a stage that is already complete.
 - Enter a number that is smaller than the number of cycles that have already been completed.
3. Select **Send** to return to the Run Monitor screen. The run continues, using the new number of cycles.

Pausing a run

1. In the Run Monitor screen, select **Pause Run**. The Pause Run dialog box is displayed:

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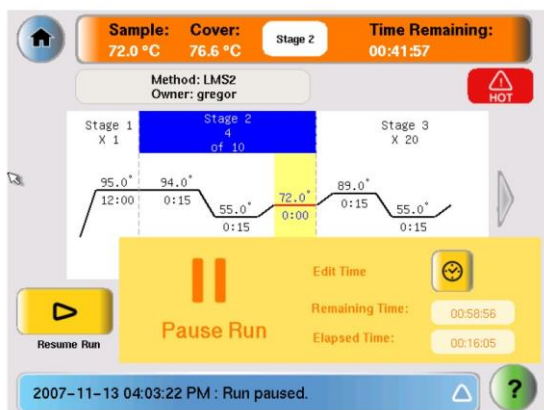
Written by: J.Bowdrey	Reviewed by:	Approved by: C.Kavanagh
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Standard Operating Procedure

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Title: Use and Maintenance of the Applied Biosystems 96 well fast thermal cycler

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The run pauses for the duration set in the Set Time-Outs. Elapsed Time is continually updated to show the run in process.

2. If you want to:

- Pause until the default pause has elapsed – Do nothing.
- Pause for a specific amount of time – Select **Edit Time**, enter the duration of the pause, then select **Done**. The run resumes when the pause is complete.
- Resume the run immediately – Select **Resume Run** (to the left of the dialog box).

Stopping a run

In the Run Monitor screen, select **Stop**, then **OK**. The run stops and the Run Report opens.

At the end of a run, the Run Report screen is displayed (below). If the last stage of the run method is an infinite hold, you must select **Stop**, then **OK** in the message box before the Run Report is displayed.

You can also see the Run Report by selecting **View Last Run** in the Tools Menu.

Removing the samples from the instrument

Grasp the sample tray/retainer assembly or reaction plate firmly with both hands and gently rock it back and forth until the tubes (or plate) are free.

CAUTION! Sample caps may pop off if the cover is opened when the sample block(s) temperature is above 27 °C.

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Cycle performance test and temperature verification kit- See user manual.

To clean the sample wells:

1. Open the heated cover.
2. Remove the sample tray from the sample block(s) and set it aside.
3. Use a cotton swab soaked in pure isopropanol to clean the sample wells thoroughly. Make certain that the isopropanol has evaporated completely before reloading a sample tray.

Decontaminating the sample wells

If the sample wells become contaminated with any biological agents, clean the wells thoroughly with a cotton swab soaked in 1:10 v/v dilution of 5.25% sodium hypochlorite (Chlorox bleach).

Use bleach solution in moderation. Applied Biosystems™ recommends the use of 10% bleach solution for removing contamination from the Veriti™ instrument sample block(s); excessive use of the solution, however, can corrode the sample block(s) material. To prevent damage to the sample block(s):


- Avoid applying excessive amounts of bleach solution. If possible, instead of using a squeeze bottle or soaked cloth, use an atomizer to deliver the solution to the wells of the block(s).
- After treating with bleach solution, rinse the sample block(s) thoroughly using deionized water.

Note: Removing residual bleach from the surfaces of the Veriti™ instrument using water minimizes the long-term effects of bleach treatments.

If the sample block(s) become contaminated with radioactivity, use a commercially available decontaminant to remove the contamination. If the block(s) cannot be decontaminated, the instrument cannot be returned to Applied Biosystems™ for service.

Cleaning the heated cover

Clean the heated cover once a month or as needed.

 **CAUTION!** During instrument operation, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block(s) can be as high as 100 °C. Before performing the procedure, keep hands away until the heated cover and sample block(s) reach room temperature.

To clean the heated cover:

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Location: CBE (H34)

1. Open the heated cover.
2. Soak a cotton swab or piece of clean cloth with pure isopropanol and gently wipe the heated platen.

WARNING! CHEMICAL HAZARD. Isopropanol is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. Exposure may cause central nervous system effects such as drowsiness, dizziness, and headache. Read the MSDS, and follow the handling instructions.

3. Remove any remaining isopropanol from the cover.

Note: If the platen becomes contaminated with amplified DNA, then raise the heated cover to the cleaning position, wipe the platen with a cloth or cotton swab soaked in bleach, then rinse with water.

Clean the heated platen once a month or as needed.

Decontaminating the heated cover

If the heated cover become contaminated with amplified DNA, raise the heated cover to the cleaning position and wipe the cover with a cloth or cotton swab soaked in 1:10 v/v dilution of 5.25% sodium hypochlorite (Chlorox bleach), then wipe the cover with a damp cloth.

For any problems see the user manual for Trouble shooting.

Predefined Run Methods.

The following run methods are supplied with the Veriti™ Thermal Cycler and are stored in the Shared folder. These run methods are based on recommended protocols from Applied Biosystems™ and should be considered as starting points for the different applications. Your results may vary between instrument models, so some optimization may be needed. Please note that the ramp rates of these methods are 100%.

- “AmpliCycle™ Seq Run Method“
- “AmpliTaq Gold™ DNA Polymerase Run Method“
- “AmpliTaq Gold™ Fast PCR Run Method“
- “BigDye™ Kit Fast Run Method“
- “BigDye™ Kit Standard Run Method“
- “Fast PCR Run Method“
- “General PCR Run Method“
- “LMS2 Run Method“

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Title: Use and Maintenance of the Applied Biosystems 96 well fast thermal cycler

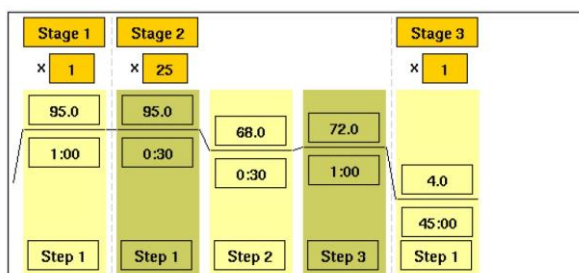
Location: CBE (H34)

- “Time Release Run Method“
- “Touchdown PCR Run Method“
- “XL PCR Run Method“

These run methods cannot be edited or deleted, but if you save them with a different name or to a different folder, you can edit them.

AmpliCycle™ Seq Run Method.

Use this run method to achieve clean sequence ladders from femtomole quantities of template. The cycle sequencing process and the benefits of AmpliTaq™ DNA™ Polymerase, CS, are described in the product insert for the AmpliCycle Sequencing Kit (PN N8080175).



AmpliTaq Gold™ Run Method

Use this run method for hot-start PCR in place of labor-intensive methods such as DNA Polymerase manual hot-start or wax-bead-mediated hot-start techniques. The hot-start technique helps to minimize the formation of primer-dimers or nonspecific products, thereby increasing specificity and sensitivity of PCR. This run method specifies a pre-PCR heat step for activation of AmpliTaq Gold™ DNA Polymerase.

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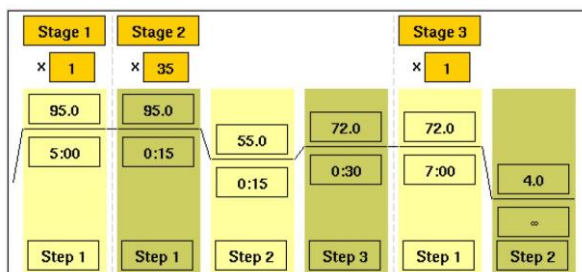
Approved by: C.Kavanagh

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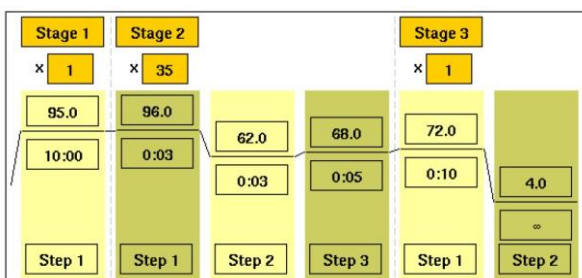
Title: Use and Maintenance of the Applied Biosystems 96 well fast thermal cycler

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AmpliTag Gold™ Method

Use this run method for PCR with AmpliTaq Gold™ Fast PCR Master Mix, UP to Fast PCR Run produce optimal yield and specificity for a 500 bp amplicon. This run method is optimized to generate template for DNA sequencing reactions and is only available™ on Veriti 96-Well Thermal Cyclers.



Note: By default, the annealing temperature is set at 62 °C. Replace this temperature with the melting temperature of the primer.

BigDye™ Kit Fast Run Method

Use this run method for cycle sequencing of dideoxy (Sanger) terminator sequencing using ABI PRISM™ BigDye™ Terminator Cycle Sequence Ready Reaction Kits.

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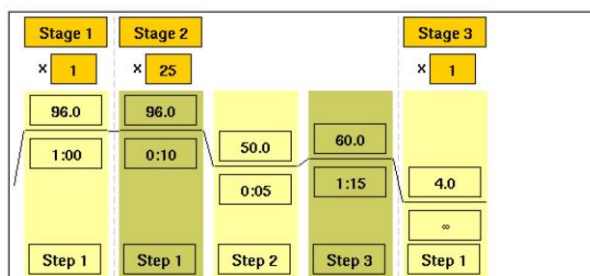
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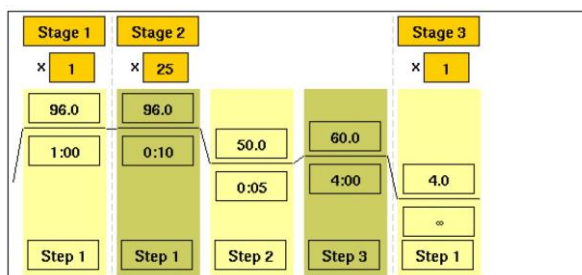
Title: Use and Maintenance of the Applied Biosystems 96 well fast thermal cycler

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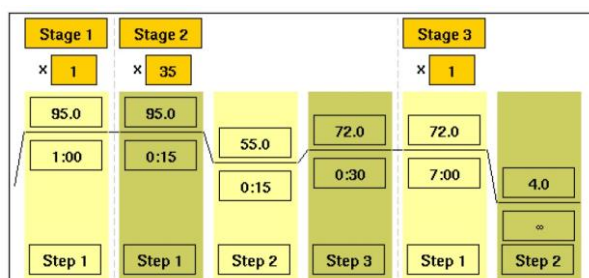


BigDye™ Kit Use this run method, which consists of cycle-sequencing parameters for dideoxy Standard Run(Sanger) terminator sequencing, with ABI PRISM™

BigDye™ Terminator Cycle Method Sequencing Ready Reaction Kits.



The pre-coded conditions in this run method are optimized for AmpliTaq™ DNA Polymerase, FS and the cycle



sequencing reagents supplied with the BigDye™ Terminator kits.

Fast PCR Run Method

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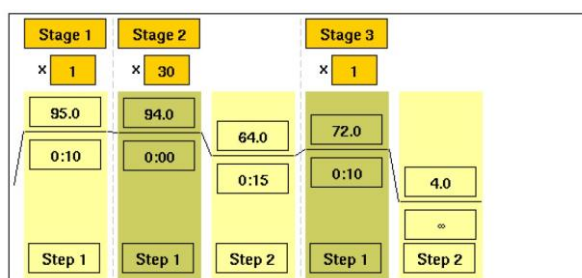
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Use this run method for use with the GeneAmp™ Fast PCR Master Mix. For more information on how to convert your standard methods to Fast methods, refer to the product literature for Fast PCR Master Mix available from www.appliedbiosystems.com.

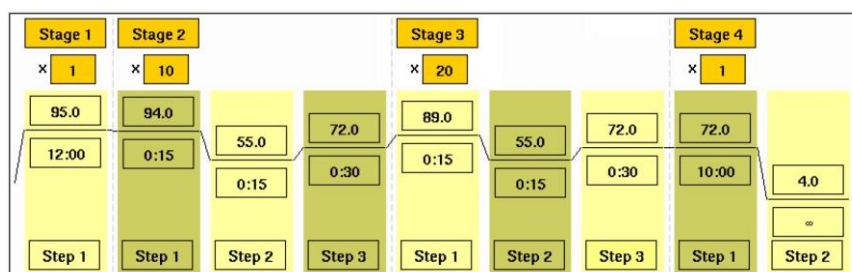
General PCR Run Method

Use this run method for standard PCR use. This method is set as the default run method for the Veriti™ Thermal Cycler.



LMS2 Run Method

Use this run method for ABI PRISM™ Linkage Mapping Set Version 2.5 (LMS2) thermal cycling. Linkage Mapping Set Version 2 uses over 400 fluorescently labeled PCR primer pairs for analysis of select microsatellite loci from the Génethon human linkage map.



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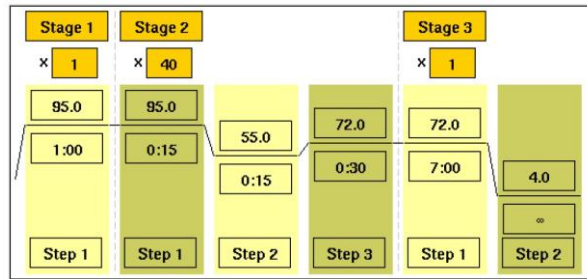
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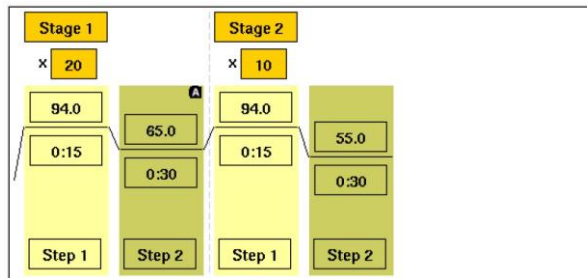
Time Release Run Method

Use this run method for use with AmpliTaq Gold™ DNA Polymerase. This method minimizes the pre-PCR activation step and adds a minimum of 10 additional cycles, allowing for slow activation of the enzyme during cycling. This provides a simple method where polymerase activity increases more slowly as product accumulates, improving specificity



Touchdown PCR Run Method

Use this run method if the optimal annealing temperature is not known. This method incrementally decreases the annealing temperature in early cycles to maximize the yield of specific products.



XL PCR Run Method

Use this run method for amplification of 5 to 40 kb PCR products using *rTth* DNA Polymerase, XL and unique reaction conditions. By providing longer templates, XL PCR complements technologies for rapid, long-range PCR. More complete genes can be amplified in one reaction from known expressed sequences, allowing more introns to be spanned. You can use XL PCR to amplify the control target (a 20.8-kb product from Lambda DNA) supplied in the kit.

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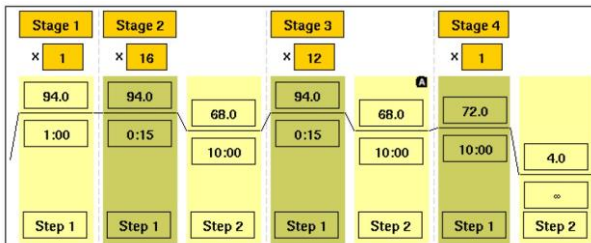
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. Equipment Malfunction

- (i) If any part of the equipment fails or malfunctions, the user should contact the Laboratory Manager. With permission of the Lab Manager the user should consult the Operator Instruction Manuals to access fault finding and troubleshooting procedures.
- (ii) All maintenance , problems and corrective actions should be recorded in the Equipment Maintenance record.
- (iii) If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the Laboratory Manager must be informed and a "Do Not Use" notice should be posted on the cytometer. Contact the manufacturer for advice and coordinate with the Lab Manager for external maintenance and servicing.
- (iv) External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (refer to SOP003 for further details) and a 'Decontamination Certificate' has been issued by the School/Building/Unit Safety Co-ordinator.

6 DOCUMENTATION

The following records are outputs of this SOP:

- QS-FORM-009 Decontamination of Equipment certificate
- Equipment Maintenance Record
-

These records will be filed in Equipment file .

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SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
		[Insert specific changes from previous SOP] < e.g. changes in accountabilities, process steps, deviation actions, or records>.	

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