Document Number:	**SASoM/EQUIP/113.v1**
Title:	**Use of the QIAamplifier 96 Thermal Cycler.**
Version:	**v1**
Author:	**Peter Mullen**

Effective from:	14/12/2021
Valid to:	14/12/2026

SOP History		
Number	Date	Reason for Change
v1	14/12/2021	Original

1.0 Purpose –

The purpose of this SOP is to outline the principles and routine use of the Qiagen QIAamplifier 96 Thermal Cycler in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the routine use and maintenance of the Qiagen QIAamplifier 96 Thermal Cycler within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the Qiagen QIAamplifier 96 Thermal Cycler within the SASoM to comply with this SOP.

4.0 Procedure –

The QIAamplifier 96 is an end-point thermal cycler. It is designed to amplify nucleic acids by repeated cycles of heating and cooling using DNA polymerases in a PCR reaction.

The QIAamplifier 96 combines modern design with user-friendly software. The user interface consists of a touchscreen with a graphical or spreadsheet display that shows the time, the status, and the temperature program for each run. Using the touchscreen keyboard, information and program parameters can be entered directly on the screen. Due to the high ramp rates, the QIAamplifier 96 thermal cycler is suitable for fast PCR applications, thereby helping to shorten program run times.

The device is controlled by a 7” touchscreen display and an easy-to-use user interface. PCR protocols and run log files can be saved to a connected USB stick. By using the USB functions, PCR protocols can be exchanged easily between devices and run log files can be stored for documentation reasons on a PC.

The QIAamplifier 96 thermal cycler features an automatic restart. If a power failure occurs during the run, the device will continue the run as soon as power is restored. In case of long-term power failure (longer than 30 minutes), the device will keep the sample block at 4°C (freeze step) and the user can decide to repeat the run with the same samples or to discard them.

The gradient function can be used for the optimization of new primer pairs. The Linear Gradient Tool allows the programming of gradients with a defined temperature difference between the rows or columns of the sample block.

This Standard Operating Procedure is NOT intended to be a full instruction manual but rather a working summary of the instrument. ALL USERS MUST THEREFORE CONSULT THE APPROPRIATE SECTION(S) OF THE INSTRUCTION MANUAL BEFORE using the instrument.

A printed copy of the User Manual for this instrument is available adjacent to the instrument.

Operation of the QIAamplifier 96:

6.1 QIAamplifier 96 front view

1 Dial for adjusting the contact pressure
2 Lid sealing button
3 Display
4 USB port

Figure 2. Front view of the QIAGEN QIAamplifier 96.

6.2 QIAamplifier 96 rear view

The Ethernet port, mains plug and mains switch are located at the rear of the QIAGEN QIAamplifier 96.

1 Ethernet port
2 Mains switch
3 Fuse holder
4 Mains plug

Figure 3. Rear view of the QIAGEN QIAamplifier 96.

The QIAamplifier 96 has a height-adjustable lid for optimal contact pressure on the samples.

6.3.1 Closing the lid

1. Place the samples in the block, then close the lid until you feel the button in the front click into position.
2. Turn the lid wheel clockwise until the clutch mechanism is activated (you will hear a clicking noise).

ATTENTION	The strength of the lid contact pressure has been designed for a fully populated block. If only a few samples are to be used in the block, place an additional two (empty) tubes of the same height in the four corner positions of the block. Otherwise the sample tube may be damaged from excess contact pressure (see also Figure 4). Never attempt to close the heated lid with force! This may damage the device.

96 Well Block

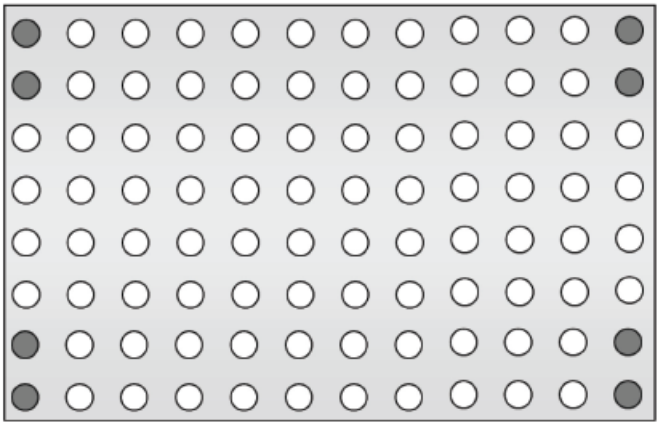


Figure 4. Placement of sample tubes in the block.

NOTE 1: ALL USERS MUST FOLLOW THE PRESCRIBED INSTRUCTIONS ABOVE WHEN SETTING UP THE INSTRUMENT / CLOSING THE LID.

6.3.2 Opening the lid

ATTENTION 	Never open the lid under pressure! Always follow the sequence below when opening the lid. If the lid is opened under pressure, the locking mechanism may be damaged.

1. Reduce the contact pressure of the lid completely by turning the lid wheel counterclockwise until there is no resistance.
2. Open the lid by pressing the button on the front of the lid.
   - **The lid opens and locks in a defined end position.**

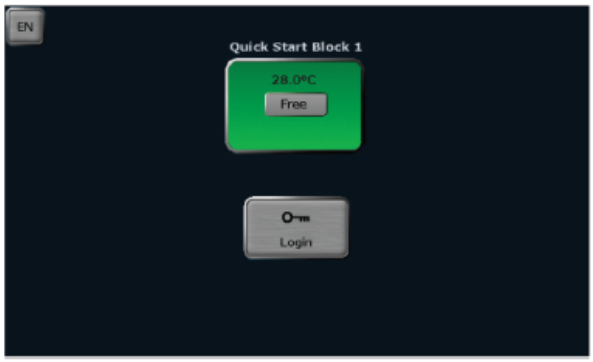
NOTE 2: ALL USERS MUST FOLLOW THE PRESCRIBED SEQUENCE OF EVENTS WHEN OPENING THE LID.

12 Quick reference guide

WARNING 	Before initial operation, check the correct operating voltage is set on the voltage selection switch on the underside of the device. Do not open the housing. Danger of electric shock!
CAUTION 	The sample block and the heated lid can reach high temperatures during operation. Both the sample block and the heated lid can burn you. The samples are heated up quickly and may come to an explosive boil. Wear goggles when handling hot samples. Make sure that the lid is closed securely before starting the program!
NOTICE 	The use of oil between samples and sample block is not necessary. If you wish to use oil, never use silicone oil. Use mineral oil. Ensure that the ventilation slits are clear. **Important**: Release the lid wheel before opening the lid! Adjust the lid wheel for every individual run.

Login

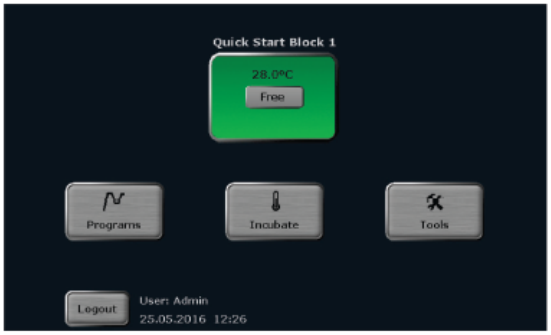
After the QIAamplifier 96 has completed the initial self-test, the login screen is displayed:



- Press **Login** to log in as an existing user.
- Press **Quick Start** for the non-user-specific quick start of programs.

Home screen

After login the QIAamplifier 96 home screen opens:



- Press **Quick Start** for the user-specific quick start of programs.
- Press **Programs** to edit, save, copy, or run programs.
- Press **Incubate** to incubate the sample block at a constant temperature.
- Press **Tools** to access the system settings, user management, documentation, backup tool, or self-test functions.
- Press **Logout** to log out.

7.5 Block status

In the login screen (see chapter 7.2) and in the home screen (see chapter 7.4), a special button with information on the current block status is shown. The sample block status can be **Free**, **Running**, or **Pause** and is indicated by the color of the button and the textbox in the middle of the button ②. In the line above the textbox, the current block temperature is shown ①. If the temperature is higher than 70°C, the characters are shown in red and the warning Hot plus the corresponding warning symbol are displayed. In the line below the textbox, the remaining runtime is shown ③. The sample block number is shown above the button.

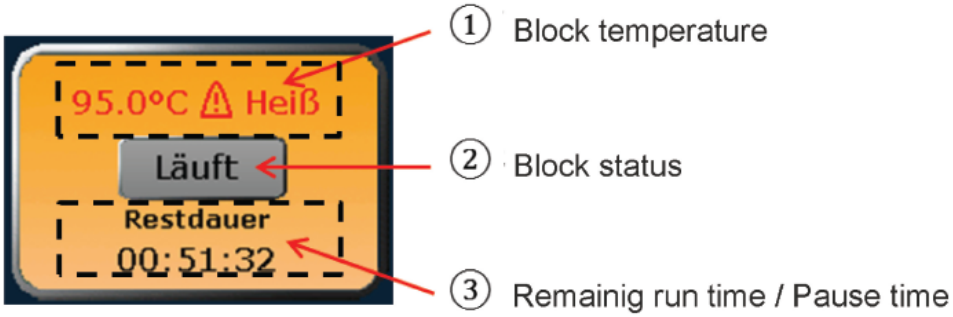
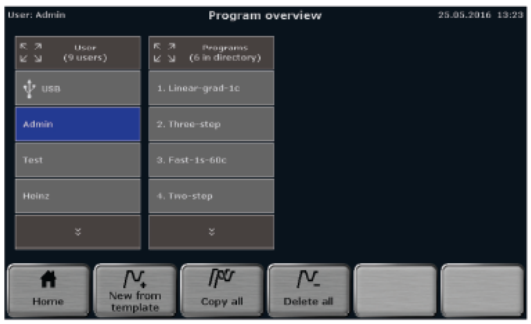


Figure 11. QIAamplifier 96 block status/quick start button.

The following table summarizes the different button colors and sample block statuses. When the button is pressed, it depends on the sample block status which function is activated in the QIAamplifier 96 software. If the status is **Running** or **Pause**, a touch of the button will lead to the program view (see chapter 10.1). If the status is **Free**, the quick start function is activated (see chapter 7.6).

Editing, saving, and copying programs

- Press **Programs** on the QIAamplifier 96 home screen. The program overview screen opens:

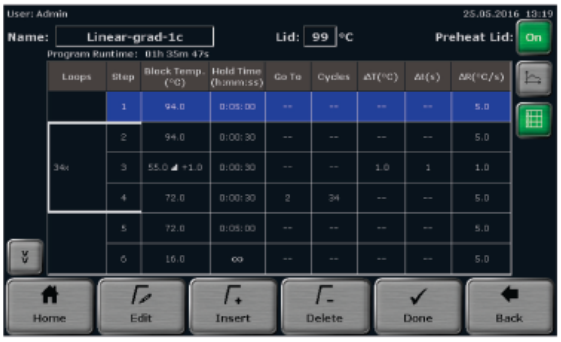


- To edit a template, press **New from template**, select a template from the list, and press **Open template**.
- To edit a program, select a user directory and a program, then press **Edit**.
- Alternatively, a user or a program can be selected via the quick selection.

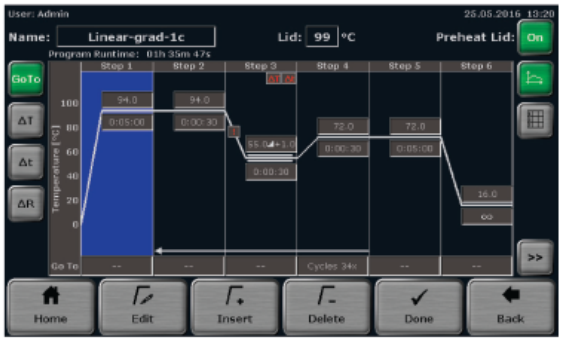
The QIAamplifier 96 programming screen opens. Use the following buttons to toggle between the different programming modes:

Graphical mode  **Spreadsheet mode**  **Gradient mode** 

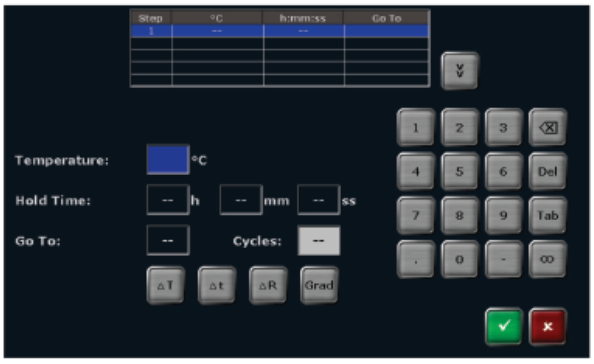
Spreadsheet programming screen



Graphical programming screen

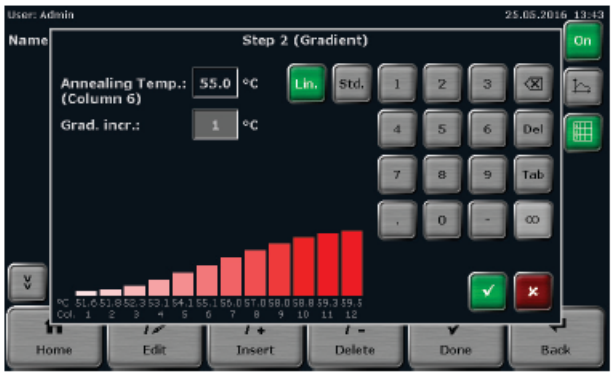


- Enter a program name. To do this, tap the **Name** field and enter a name using the QIAamplifier 96 keyboard.
- Define the status and the temperature of the heated lid. Tap the **Heated lid** field and switch the heated lid on or off. If the heated lid is switched on, enter a temperature between **30°C** and **110°C**.
- To switch the heated lid preheating on or off, press the corresponding button.
- Select a program step and press **Edit**. The screen for editing program steps opens:



- Use the cursor keys above the keyboard to switch between program steps. The number of the currently activated step is displayed on a blue background in the table at the top of the screen.
- Tap the **Temperature** field and enter a temperature in **°C**.
- Tap the **Hold time** field and enter a time using the **h:mm:ss** format. To program a pause step, press ∞.
- To program a loop, tap the **Go To** field and enter the step number to which the program should return. Then tap the **Cycles** field and enter how often the loop should be repeated.
- To reduce or increase the annealing temperature from step to step within a loop, press **ΔT** and set a negative or positive temperature increment.

- To reduce or increase the hold time from step to step within a loop, press **Δt** and set a time increment.
- To adjust the heating rate, press **ΔR** and enter a value between 0.1°C and max. You can accept the heating rate for all steps in the program by checking the **Apply heating/cooling rate to all steps** box.
- To program a gradient, press **Grad**. On the next screen, you can choose between standard **Std.** and linear gradient programming **Lin.**. For standard programming, enter a temperature for both the first and last row in the sample block. For linear programming, enter the primer annealing temperature and set a temperature increment. The gradient curve is displayed graphically. Press ✓ to confirm your entries.



- Press ✓ on the screen for editing program steps to accept the settings. The software leaves the screen and opens the spreadsheet or graphical program display.
- Alternatively, individual program parameters for a step can also be edited. To do this, in the spreadsheet view press directly on the cell for the parameter in the program table which you want to edit. In the graphical view press on the desired parameter edit field. Program options (loops, temperature, and time increment, as well as heating and cooling rate) must be activated using the button to the left of the graphical display.
- To insert a program step, select a step and press **Insert**. The screen for editing program steps opens. Enter the desired parameters and confirm the settings by pressing ✓.
- To delete a program step, select it and press **Delete**.

Incubation at 15°C rather than 4°C at the end of a run increases the device's life time.

- When all the parameters for all steps have been entered, confirm the settings on the programming screen with **Done**. The software opens the screen for copying and storing programs.
- To save the program, select a user directory and a storage location. Then, press **Save**. The program is now saved.

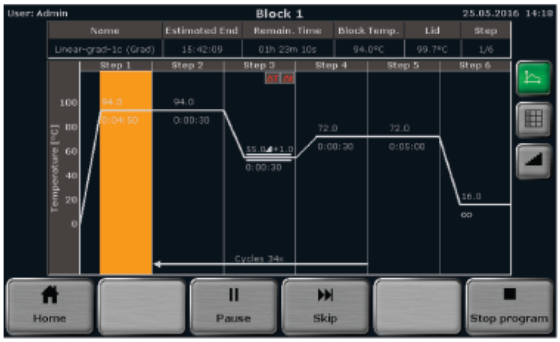
- To copy programs, press **Programs** on the QIAamplifier 96 home screen.
- To copy all programs located in a directory, select a directory and press **Copy all**. Select a directory to which the programs should be copied and press **Save**.
- To copy a single program, select a directory and a program and press **Copy**. Select a directory and a storage location and press **Save**.
- To delete all programs located in a directory, select it and press **Delete all**. Confirm the confirmation prompt.
- To delete a single program, select a directory and a program and press **Delete**. Confirm the confirmation prompt.

Starting, stopping, and pausing programs

- The QIAamplifier 96 stores the latest 5 started or edited programs for each user. To quick start a program, press **Quick Start** on the QIAamplifier 96 home screen. Select a program from the list and press **Start**.
- To start a program from a user directory, press **Programs** on the QIAamplifier 96 home screen. On the next screen, select a directory and a program and press **Start**.
- After the start, the current program is shown in a spreadsheet, graphical, or gradient view. Use the same buttons as in the programming screen to switch between the different views. The active step is highlighted in yellow:

Spreadsheet view

Graphical view



- To pause an active program, press **Pause**. The color of the highlighted step changes to blue and the message **Pause** is shown in the **Step** field.

- To continue a paused program, press **Continue**.
- To skip a step, press **Skip**. The program will continue with the next step.
- To stop an active program, press **Stop program** and confirm the confirmation prompt. Programs with a pause in the last step have to be stopped manually as well.

Cleaning and Maintenance:

The exterior of the device may only be cleaned with a damp, not dripping wet, cloth after the device has been switched off.

Do not use alcohol (e.g., methanol or ethanol), organic solvents, or abrasives to clean the device.

Any maintenance on the device may usually only be carried out in the switched-off condition (unless stated otherwise).

 Observe the following:

- Free access to the power switch on the back of the enclosure has to be ensured during operation.
- The ventilation fittings at the rear of the device must be unobstructed and operational. Covered ventilation grilles or slits etc. may cause the device to break down or may cause damage to it.
- The use of oil between the samples and the sample block is not necessary to achieve an improved heat exchange. However, if you still want to use oil, you should use mineral oil. Do not use silicone oil.
- Be careful to avoid crushing or pinching injuries when closing the device.

 The QIAamplifier 96 may cause burns. Observe the following:

- The thermal block, the samples and the heated lid reach high temperatures. There is a risk of burns during contact.
- Ensure that the lid is securely closed before starting the program. Do not touch the heated lid.

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- Ensure that the lid is securely closed before starting the program. Do not touch the heated lid.

- The rapid heating of the thermal block can cause liquids to boil explosively. Always wear safety goggles during operation.
- Do not touch hot sample tubes or plates and do not open them or boiling liquid may escape.
- Only use plates and tubes that are suitable for high temperatures (up to 100°C), fit well into the thermal block (no shaking), and whose lids seal tightly.

The following must be observed:

- The exterior of the device may only be cleaned with a damp, not dripping, cloth after the device has been switched off.
- Do not use alcohol (e.g., methanol or ethanol), organic solvents, or abrasives to clean the device.
- Any maintenance on the device may usually only be carried out in the switched-off condition (unless stated otherwise).

5.0 Personal protection –
Howie coat must be worn at all times.

6.0 Training –
All users have to be trained by a designated person before using the Instrument.

7.0 Related documents –
Please read the online manual for further information.

8.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date: 14/12/2021
Management Approval:		
Name:	Peter Mullen	
Position:	SOP Administrator	
Signature:		Date: 14/12/2021
QA release by:		
Name:	Claire Sneddon	
Position:	QA Manager	
Signature:		Date:15/12/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE