

Thermocycler PCR Run/Operation Protocol

(Eppendorf AG 22331 Hamburg)

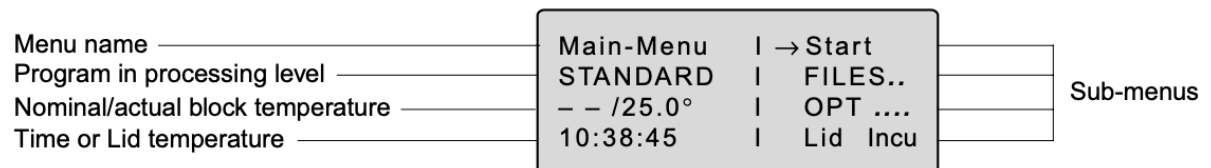
For more detailed info about menu, programs, device set-up, etc - see manuals:

- <https://arboretum.harvard.edu/wp-content/uploads/2020/07/Eppendorf-MasterCycler-Manual.pdf>
- <https://uh.edu/tech/clist/documents/manuals/standalone-equipment/epgrad.pdf> **

The **Thermal Cycler** (also known as a **Thermocycler**, PCR Machine or DNA Amplifier) is a laboratory apparatus used to amplify segments of DNA via the Polymerase Chain Reaction (PCR). ... The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps.

0. Switching on the device

- Press the main power switch on the rear of the device. The name and current software version of the device will be shown briefly on the display.
- The main menu will appear on the display. For further detail on each menu component, see manual:



1. PREPARATION

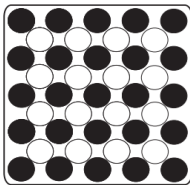
1.1 Sample loading

In order to achieve an optimum temperature transition, it should be ensured that the tubes sit correctly in the Thermoblock and do not wobble. Tubes that are not sufficiently temperature resistant (to app. 120 °C) may not be used.

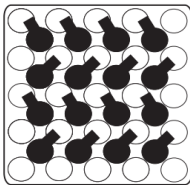
This generally also applies for foils for sealing PCR plates. Due to the controllable lid heating it is also possible to use foil that melts at over 100 °C. The lid heating could be set to 95 °C for example.

The Mastercycler ep gradient and Mastercycler ep gradient S can be loaded with up to 96 PCR tubes (0.2 ml) or one 96-well PCR plate. In order to guarantee even pressure of the heated lid when using tubes, we recommend positioning a tube in each corner of the Thermoblock. Additional tubes should be evenly distributed in the block.

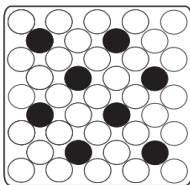
Placement example:



25 x 0.2 ml PCR test tubes
or
microtiter plates (5x5)



16 x 0.5 ml thin-walled PCR test tubes
(special tube from Eppendorf)



8 x 0.5 ml Eppendorf Safe-Lock micro test tubes
0.5 ml tubes with wide lid, e. g. 0.5 ml Eppendorf
micro test tubes, should be loaded in a staggered way

*The Mastercycler ep 384 can be loaded with a 384-well PCR plate.

1.2 Sample volume

The temperature control can be set to the Thermoblock or to the tubes and sample quantities used. In the program header of each PCR program it is possible to choose between the temperature modes Block, sim Tube and active Tube under the menu point Control. The adjustment to the tubes and sample volumes takes place at the start of the program.


The sample quantity should then be:


- 0.2 ml PCR tubes and 96-well PCR plates - between 10 µl and 100 µl
- 384-well PCR plates- between 5 µl and 25 µl.

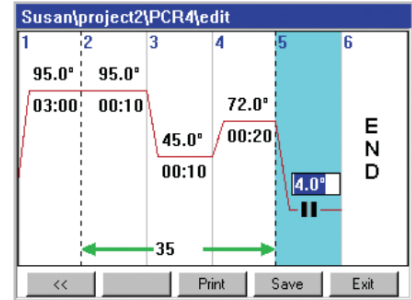
2. START PROGRAM

A logged-on user can start a created PCR program in a variety of ways.

From the editor:


 Save the generated program.
Insert the sample tube.
Draw the ESP heated lid forwards over the samples (only necessary for manual operation).


 Start the program.





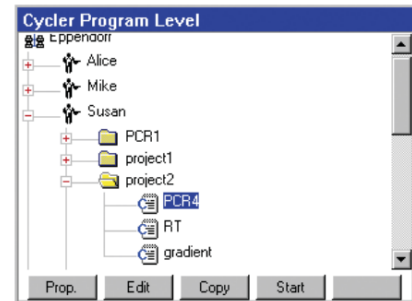
In order to attain optimally distributed pressure when using only a few sample tubes, these should be inserted in the middle and empty tubes in the four corners of the Thermoblock.

In the navigation tree:



 Move mark to the program.





 Insert sample tube.
Draw the ESP heated lid forwards over the samples (only necessary for manual operation).




 or  Start the program.

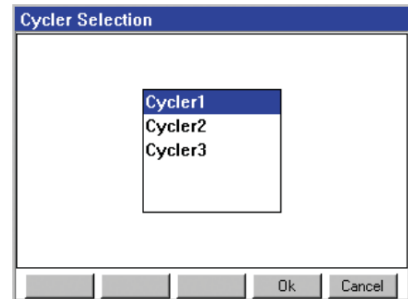


The following input should be made after the program is started:

  Select a connected Thermocycler (only with several connected devices).

    Select of the tube type used

 to   Input of the filling volume (only for Control: sim Tube).



The program starts and the status display appears after this input is made.

3. STATUS DISPLAY

The status display appears immediately following the start of a program. The progress of a running program can be followed in the status display. Current program information is shown. The presently active step is recognized as a blinking line in the graphic display. In addition to the graphic display, program-specific information is shown in two information boxes.

The screenshot shows a status display window titled "Program... \Susan\NEW on Cyclor 1". It features a graphical timeline with temperature and time data for six steps. Below the graph are two information boxes: one for runtime and device status, and another for cycle and temperature details. A green bar at the bottom indicates the current status is "Running". At the very bottom are buttons for "Stop", "Abort", and "Exit".

Step	Temp (°C)	Time
1	94.0°	00:15
2 Cyc 1	94.0°	00:15
3 Cyc 1	55.0°	00:15
4 Cyc 1	72.0°	00:30
2 Cyc 2	94.0°	00:15
3 Cyc 2	55.0°	00:08

- 1 Program path and cyclor
- 2 Program step
- 3 Remaining run time
- 4 Start time and end of the program
- 5 Current block and heated lid temperature
- 6 Status of the device
- 7 Cycle number
- 8 Nominal temperature
- 9 Remaining holding time
- 10 Information of the current program step

3.1 Exit the status display

The status display can be left with Exit.



3.2 Calling up the status display



Mark the desired Mastercyclor ep.



Call up the status display.

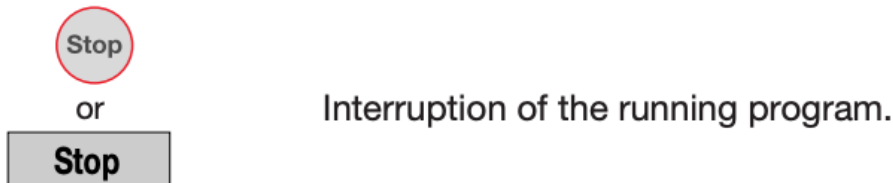


4. FUNCTIONS IN THE STATUS DISPLAY

The following functions in the status display are possible while a program is running:

4.1 Interruption under a running program

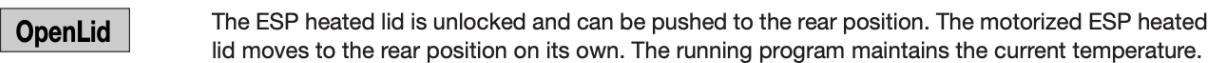
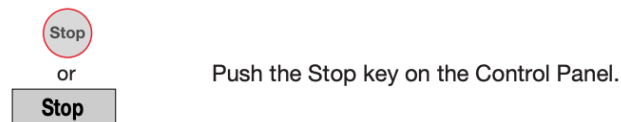
The program can be interrupted, for instance, in order to add reagent.



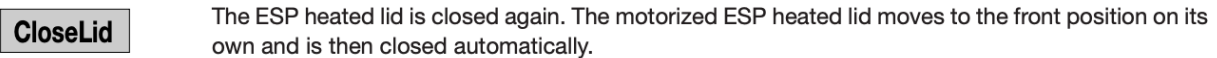
The current temperature of the block and the lid is maintained and the colored status display changes to yellow and shows paused.

4.2 Opening and closing the ESP heated lid

The heated lid can be opened as follows while the program is running.



If the program is then resumed, the ESP heated lid is again pulled forward over the samples.



4.3 Continue program

A stopped program can be continued at any time.



The program is continued at the point of interruption and the colored status display changes to green again and shows running.

4.4 Abort program

A running program can be aborted at any time.



Program "idle" appears in the display.

5. PROTOCOL

For purposes of documentation following the run of a PCR program, the affiliated protocol can be saved either on the MMC™ or printed on a connected printer.



Move mark to the desired program under the Mastercycler ep.

Protocol

Invoke the protocol.

Print

Print out on a connected printer.

Export

Save the protocol on a MMC™.

Note: The next start of a program on the Mastercycler ep results in the deletion of the protocol and the creation of a new one.

6. REMOVE FROM OPERATION

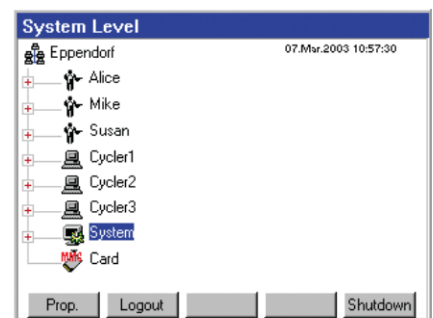
The system must be shut down in order to switch off the Mastercycler ep



Move mark to the Eppendorf System or Card node.

Shutdown

Shut down the system.



Following the shutdown it is possible to switch off all connected Mastercycler ep with the power switch.