

# Mastercycler<sup>®</sup> / Mastercycler<sup>®</sup> gradient

Bedienungsanleitung · Operating Manual · Mode d'emploi Instruzioni d'impiego · Manual de Instrucciones



# Certificate

## Authorized Thermal Cycler

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, is an Authorized Thermal Cycler.

Its purchase price includes the up-front fee component of a license under the foreign counterparts of U.S. Patents Nos. 4,683,202, 4,683,195 and 4,965,188, owned by F. Hoffmann-La Roche Ltd, covering the Polymerase Chain Reaction ("PCR") process, to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. No rights are conveyed expressly, by implication or estoppel to any patents on real-time methods, including but not limited to 5' nuclease assays, or to any patent claiming a reagent or kit.

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Applied Biosystems does not guarantee the performance of this instrument.

Bedienungsanleitung 1
<i>Operating Manual</i>
<i>Mode d'emploi</i>
Istruzioni d'impiego
<i>Manual de Instrucciones</i>
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# 1 Introduction

The Eppendorf Mastercycler<sup>®</sup> and Mastercycler<sup>®</sup> gradient are PCR devices for molecular biological and biochemical research laboratories.

Temperature control is carried out with Peltier elements, which enable extremely rapid changes in temperature of up to 3 °C per second. This allows the temperature of the samples to be controlled extremely rapidly between 4 °C and 99 °C.

A special "tube control" mode has been developed for temperature-controlling samples in various different tubes with various different filling volumes.

The thermoblock has been constructed as a universal block which can accommodate microtiter plates in the 8 x 12 format, 96 Eppendorf PCR test tubes of 0.2 ml, 77 thin-walled Eppendorf PCR test tubes of 0.5 ml or other suitable tubes – without the block having to be exchanged.

With the aid of the in situ Adapter, in situ reactions may be carried out on up to four glass slides.

To prevent the formation of condensation, the devices have a heated lid which has been perfectly tailored to suit all tubes.

The devices are easy to operate, with user guidance available in the integrated, eight-line display. Experiments and tests can be documented with the aid of a printer which may be connected up to the devices.

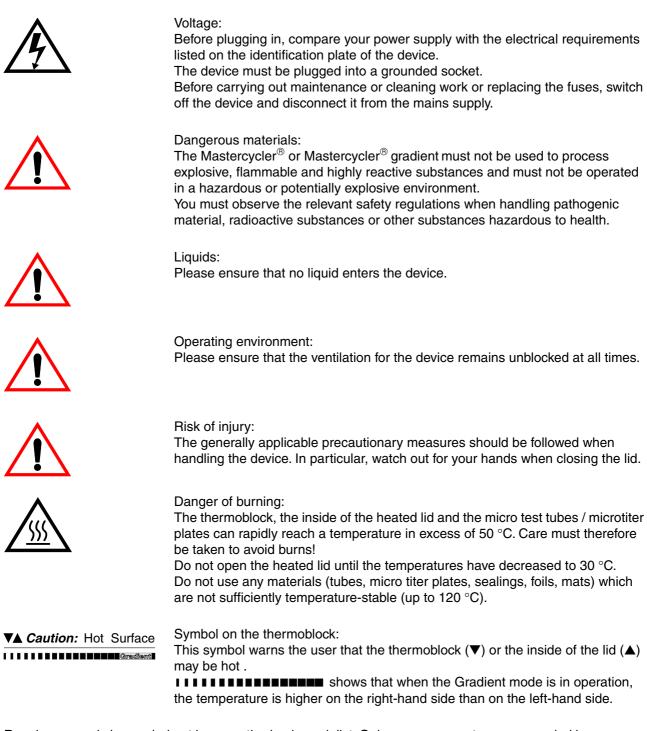
One particular advantage of the Mastercycler<sup>®</sup> gradient is the Gradient function, which greatly facilitates the optimization of PCR experiments. The gradient enables the temperature across the entire block to be varied within a range of up to 20 °C; it can be programmed with every temperature step.

The Mastercyclers are authorized cyclers licensed by the Perkin-Elmer Corporation.

# 2 Safety precautions

Before using the Mastercycler<sup>®</sup> or Mastercycler<sup>®</sup> gradient for the first time, please read the complete operating manual. The Mastercycler<sup>®</sup> or Mastercycler<sup>®</sup> gradient may be used only for performing PCR and related methods in a research laboratory.

It is essential to observe the following safety precautions:



Repairs may only be carried out by an authorized specialist. Only use spare parts recommended by Eppendorf.

#### Transfer

If the device is passed on to someone else, please include the instruction manual.

#### Disposal

In case the product is to be disposed of, the relevant legal regulations are to be observed.

The disposal of electrical devices is regulated within the European Community by national regulations based on EU Directive 2002/96/EC on waste electrical and electronic equipment (WEEE).

According to these regulations, any devices supplied after 13.08.05 in the business-to-business sphere, to which this product is assigned, may no longer be disposed of in municipal or domestic waste. They are marked with the following symbol to indicate this.



As disposal regulations within the EU may vary from country to country, please contact your supplier if necessary.

## 3.1 Delivery package

The delivery package contains the following items:

- 1 Mastercycler<sup>®</sup> or Mastercycler<sup>®</sup> gradient
- 1 Main power cable
- 1 Operating manual
- 1 Personal card
- 1 bag of 0.2 ml PCR test tubes (100 pieces)
- 1 bag of 0.5 ml PCR test tubes (100 pieces)

## 3.2 Setting up the device

When setting up the device, please ensure that enough space is available to allow the ventilation slit to remain uncovered and to allow air to flow under the device for cooling purposes. Please ensure that no objects are under the device (e.g. lab bench paper).

No special equipment is required for transporting the device. It can be lifted up and carried by being held on both sides.

Space requirements:	Width: Depth: Height:	41 cm	
Mains connection:	1 shockproof socket for the Mastercycler <sup>®</sup> / Mastercycler <sup>®</sup> gradient. If a printer is to be connected up, a second mains connection must be used.		
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The delivery packaging should be stored in a safe place to enable the device to be shippeded in the event that repairs are necessary.

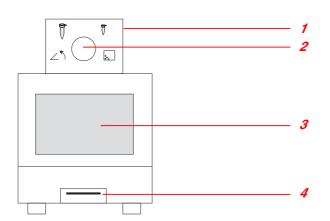
## 3.3 Starting up the device

Remove the adhesive strip on the heated lid and take out the bubble-wrap from below the heated lid.

The Mastercycler<sup>®</sup> / Mastercycler<sup>®</sup> gradient is connected to the mains supply using the mains cable. Before starting up the device, please compare the power supply with the voltage requirements listed on the identification plate (see Sec. 4.1, Fig. 3).

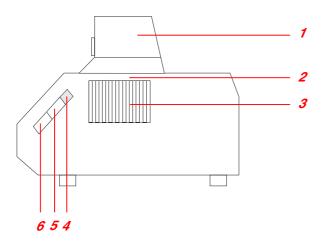
The procedure for connecting and starting up a printer is described in Sec. 10.1.

## 4.1 Device construction



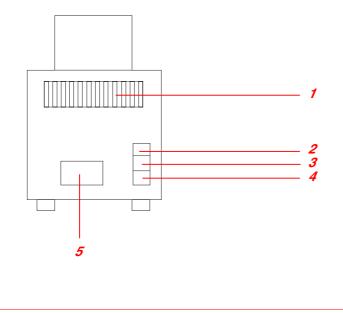
#### Fig. 1: Front view

- 1 Heated lid
- 2 Locking button
- 3 Display and control panel
- 4 Personal card reader



#### Fig. 2: Side view

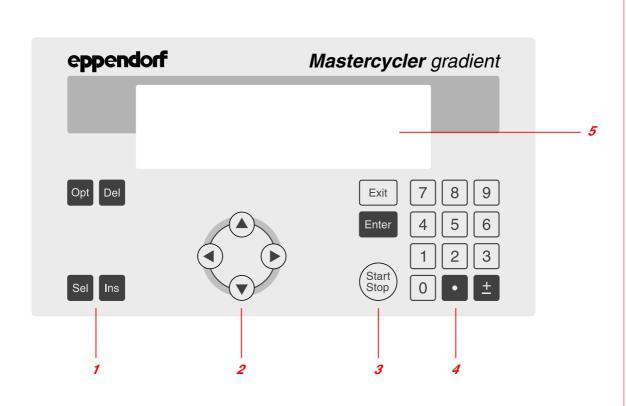
- 1 Heated lid
- 2 Thermoblock
- (not visible in this picture)
- 3 Ventilation slit
- 4 Brightness regulator
- 5 PC connection socket
- 6 Printer connection socket



#### Fig. 3: Rear view

- 1 Ventilation slit
- 2 Main power switch
- 3 Fuses
- 4 Main power socket
- 5 Identification plate

# 4 Technical description



#### Fig. 4: Display and control panel

- 1 Programming keys
- 2 Cursor keys
- 3 Control keys
- 4 Digit entry block, decimal point and reverse sign key
- 5 Display

## 4.2 Keys

#### Programming keys

- When this key is pressed, the approximate remaining run time of a program and the time Opt of the expected end of the program appears in the display for approximately five seconds. To select programming options for the "Temperature" command (ramp, gradient, increment). - To delete program lines, digits or letters or to reset parameters. Holding down the key Del deletes not only individual letters but also a complete name. - To select a program command or to select from a list. Sel - A menu item can be selected using the  $s_{el}$  key instead of the  $(\mathbf{k})/(\mathbf{v})$  keys. The sel key can also be used to select from a list and to enter the letters of program \_ names. The direction of selection of the letters can be changed by pressing the  $\pm$  key. Ins - To insert program lines during the creation of a program.

# 4 Technical description

#### Cursor keys

The cursor appears in the display as a dark field.

If a menu should be selected, the cursor must be moved to that menu and the selection should then be confirmed by pressing  $\Box$ .

The cursor is also used to move into, or to change between, input fields. Entries do not have to be confirmed by pressing  $\begin{bmatrix} Enter \\ \end{bmatrix}$ ; they are stored immediately.

#### Control keys

Exit	

- To exit a menu or to return to the next-highest menu level.

- After a program has been started, the display of the program can be exited, e.g. in order to work in the programming level.

Enter

To call up a menu which has been selected using the cursor.

To confirm an entry.

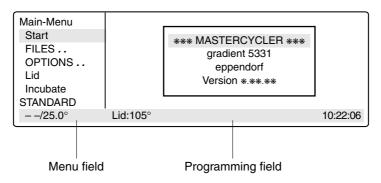


- To quick-start a program from the processing level ("FILES/Edit" menu). If another program needs to be started, the "Start" menu must be used.
- To cancel or to interrupt a program.

## 4.3 Display

Press the main power switch on the rear of the device (Sec. 4.1, Fig. 3.2). The device name and the software version installed appear briefly in the display.

The main menu then appears in the display:



The eight-line display is divided into two areas. The menu field on the left-hand side contains a list of the selectable programs. The programming field is used for programming and for setting the device parameters.

# 5 Description of menus

## 5.1 Main menu

Main-Menu — Start — FILES OPTIONS			— Menu name — Sub-menus
Lid — Incubate STANDARD — – –/25.0°	Lid:105°	10:22:06	— Program in processing level — Time
			<ul> <li>Lid temperature (only when lid is switched on)</li> </ul>
			- Nominal/actual block temperature

A menu or a sub-menu can be called up in three different ways:

- 1. Select using the ()/() keys and confirm by pressing  $E_{\text{nter}}$ .
- 2. Select using the  $(s_{el})$  key and confirm by pressing  $(E_{nter})$ .
- 3. Select the menu using the internal position number (shown in brackets in the text) and confirm by pressing Enter.

The program in the processing level can be started immediately by pressing the  $\begin{bmatrix} Start \\ Stop \end{bmatrix}$  key. Menus are exited by pressing the  $\begin{bmatrix} Exit \\ Exit \end{bmatrix}$  key. To access the main menu, it may be necessary to press the  $\begin{bmatrix} Exit \\ Exit \end{bmatrix}$  key several times. Menus written in capital letters have additional sub-items. For ease of reference, the menu name appears on the left-hand side of the display or as a headline above the menu or as a sub-item.

Overview of the entire menu structure:

Main-Menu				
Start	(1)			To start programs
FILES	(2) Edit Load Standard New Delete	(1) (2) (3) (4) (5)		To program To change a program in the processing level To load and change any program Example program To create new programs To delete programs
- OPTIONS	(3) Editor Printer Gradient GENERAL	<ul> <li>(1)</li> <li>(2)</li> <li>(3)</li> <li>(4)</li> <li>Clock</li> <li>Remote</li> <li>Sound</li> <li>Etc</li> </ul>	(1) (2) (3) (4)	System settings To display additional functions To connect a printer To display the temperature distribution To set the clock To connect a PC Tone
— Lid	(4)	VALID	(5)	To test the device To preheat the lid
Incubate	(5)			To preheat the thermoblock and the lid

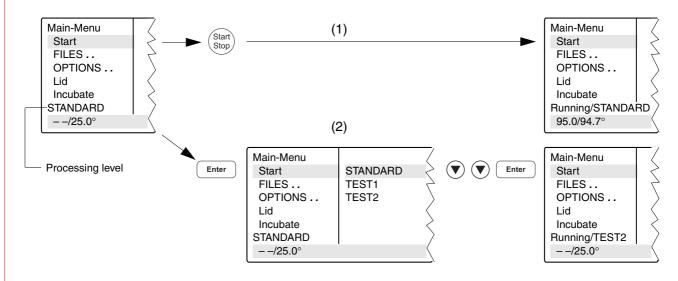
## 5.2 Start

Only programs can be started from the "Start" menu.

The program displayed in the processing level can be started directly by pressing the  $\binom{Start}{Stop}$  key (1).

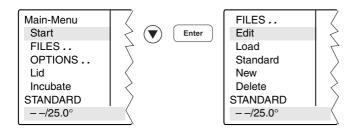
If a program which has been stored has to be started, it is selected with the cursor keys from the displayed list after  $\boxed{Enter}$  has been pressed (2) and cofirmed by pressing  $\boxed{Enter}$ .

The "Start" menu has no sub-programs.



## 5.3 FILES

The "FILES" menu is used to change, load, create or delete programs.



## 5.3.1 Edit

The "Edit" menu is used to change existing programs. The program which is to be changed may already be in the "processing level" or it is loaded into the processing level via the "Load" menu. This is applicable for programs from the internal device memory or from a personal card.

The program which has been changed can be checked by pressing the  $\binom{\text{Start}}{\text{Stop}}$  key after the program has been saved under a new name. The processing level is not deleted when this program is saved. The program data remains stored even when the device is switched off and is overwritten only when the "New" or "Standard" sub-menu is called up.

*Note:* If the program which has been changed (without being saved under a new name) is started, there are then two different programs with the same name (one in the internal memory and one in the processing level).

#### 5.3.2 Load

"Load" is used to call up a list of existing programs. These programs may be taken from the internal memory or from a personal card. The selected program is loaded into the "Edit" processing level. If the "Edit" processing level contains a program which has not been saved, a safety check appears in the display, which then enables the program to be saved.

If the user does not want to save the program, it is overwritten when the new program is loaded. As the "Edit" processing level is called up automatically, the program can be changed immediately and can then be tested for test purposes.

The procedure for loading programs from a personal card is described in Sec. 9.3.

#### 5.3.3 Standard

An example program called "Standard" is available for creating new programs. It is not necessary to rewrite a program completely; the example program can be supplemented or changed and then saved under a new name. All programs which are in the internal memory can be used as an example program.

Following a safety check, the example program is loaded into the processing level. The process is described in more detail in Sec. 7.4.

Although the "Standard" example program cannot be deleted, it can be changed to suit individual requirements. After the changes have been made, the example program is saved under the name "Standard". To restore the original example program, the "Standard" program must be deleted in the "Delete" sub-menu. When "Standard" is called up again, it is the original example program which is then loaded into the processing level.

#### 5.3.4 New

"New" can be used to call up the processing level automatically. To create a new program, an existing program is deleted.

The essential input CNTRL BLOCK, Lid =0°, NOWAIT AUTO, end is simulated automatically and the program is given the provisional title UNNAMED.

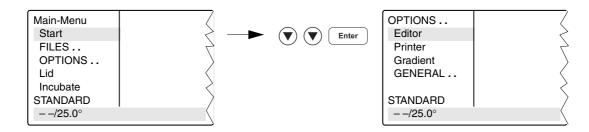
#### 5.3.5 Delete

Programs can be deleted from the internal memory or from a personal card. After "Delete" has been selected, a list of program names appears in the display. A program can be selected from this list using the cursor key and can be deleted by pressing  $\boxed{Enter}$ . The program in the processing level cannot be deleted.

The procedure for deleting programs from a personal card is described in Sec. 9.3.

## 5.4 OPTIONS

The "OPTIONS" menu can be used to define general system settings for the program editor, printer, time/date, etc.



Selection of YES/NO, ON/OFF and other settings can be carried out using the (sel) key.

The menu is exited by pressing the Exit key.

5.4.1 Editor

OPTIONS Editor	Editor Mod	le	
Printer Gradient GENERAL	Show Ext. Steps:	YES	
STANDARD /22.0°			

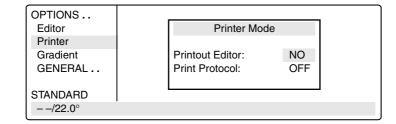
Show Ext. Steps To display temperature options.

*YES* All options (temperature increment, time increment, ramp, ramp increment, gradient) for each step appear in the display.

**NO** Only the nominal temperature and the cycle time appear in the display. If the options have been defined, the command is marked with an "\*" for information purposes when the program is called up. Nonetheless, the options can be called up by pressing the Optikey and can then be entered or displayed.

# 5 Description of menus

#### 5.4.2 Printer



Entries are necessary only when a printer is connected.

#### Printout Editor

YES:	The program in the "Edit" processing level is printed out. If "YES" has been selected, but no printer is accessible, the error message "Printer: No Response" appears in the display.
NO:	Appears automatically when the program printout has finished.
Print Protocol	
ON:	When the program starts, the commands are printed out; during the run, every completed command is printed out together with the time.
OFF:	No printout is made.

#### 5.4.3 Gradient

The Gradient function can be selected with the Mastercycler<sup>®</sup> gradient only.

The Gradient function can be used to vary the temperature distribution across the block.

In every temperature step, the thermoblock can be programmed so that each column has a different temperature. The lowest temperature is in the cavities on the left-hand side. It then rises gradually across the block so that the highest temperature is on the right-hand side.

*Show Gradient* The "Show Gradient" menu can be used to show which temperature is in which column:

OPTIONS	Show Gradient		
Editor	Tube: 0.2 ml	T=60.0	G=7.0 °C
Printer			
Gradient			
GENERAL			
STANDARD			
<i>−−</i> /22.0°			10:22:06

The tube type "0.2 ml, 0.5 ml, 0.5 ml thin, plate" is selected by sel and confirmed by pressing Enter.

After a temperature and a gradient have been entered, the temperature distribution across the block appears in the display:

OPTIONS		Show Gradien	t
Editor	Settings:	Tube: 0.2 ml	T=60.0C G= 7.0C
Printer	Pos. 1: 52.9C	Pos. 5: 56.9C	Pos. 9: 64.3C
Gradient	Pos. 2: 53.2C	Pos. 6: 58.7C	Pos:10: 65.8C
GENERAL	Pos. 3: 54.0C	Pos. 7: 60.6C	Pos.11: 66.9C
	Pos. 4: 55.2C	Pos. 8: 62.5C	Pos.12: 67.5C
STANDARD		Print Results <of< td=""><td>PT&gt;</td></of<>	PT>
<i>−−</i> /22.0°			10:22:06

The gradient may be a maximum of  $\pm$  10 °C with a resolution of 0.1 °C. This means that the overall temperature span is 20 °C. The selected temperature is in the middle of the block.

The left-hand side is temperature-controlled to nominal temperature – gradient The right-hand side is temperature-controlled to nominal temperature + gradient.

# 5 Description of menus

### 5.4.4 GENERAL

To enter general settings and to test the device.

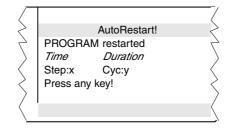
GENERAL	
Clock	
Remote	4
Sound	
Etc	
VALID	
<i>−−</i> /22.0°	
	2

Clock	Format:		e in the 24-(military) or 12-hour-clock format (choice of PM or AM) can be l using the set key.		
	Time:	To enter	the current time in hours:minutes:seconds.		
	Date:		ter the current date as day/month/year. nonth can be selected using the $sel$ key.		
Remote	Baud rate:		transmission rate of 19,200, 9,600, 4,800, 2,400 or 1,200 for a C connection can be selected using the $sel$ key.		
Sound	KeyClick:	ON: OFF:	Every keyclick is confirmed by an acoustic signal. The acoustic signal for keyclicks is switched off.		
	Warnings:	ON: OFF:	In addition to the warning text, warnings are acknowledged by an acoustic signal. No acoustic signal is emitted to acknowledge a warning.		
			<i>Note:</i> Errors are always acknowledged by an acoustic signal, irrespective of this setting.		
Etc	Start with:	MAIN: LAST:	After the device has been switched on, the main menu appears in the display. After the device has been switched on, the most-recently used menu appears in the display.		
	Auto Restart:	NO:	A program interrupted by a power failure is not continued.		
			Attention! PROGRAM interrupted <i>Time Duration</i> Step:x Cyc:y Press any key!		

YES: Depending on the duration of a power failure, an interrupted program will automatically continue or can be manually continued or stopped.

#### Power failure < 3 min:

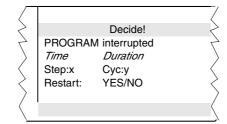
A currently running program automatically continues if power is restored within 3 minutes of the commencement of the power failure. A display indicates when, how long and at which step the program was interrupted:



When any key is pressed, the display jumps back to the program view. If the program is to be cancelled, press the (start) key and confirm by (enter).

#### Power failure > 3 min:

In the event of a longer power failure, an aucistic warning signal is given as soon as the power supply returns, and the program can be manually continued or stopped.



If the interrupted program is to be continued, select "YES" with sel and confirm with Enter. Press any key to return to the program view.

If the program is to be discontinued, select "NO" with  $\fbox{sel}$  and confirm with  $\fbox{Enter}$  .

*VALID*.. These programs can be used to test the device. The measurements can be documented by connecting a printer to the device.

The "Validate" and "Calibrate" menus require the temperature-validation system (order no.: 5331 222.005). This system consists of a special temperature sensor and a personal card with the corresponding software.

For precise information on operating the "Validate" and "Calibrate" programs, see the operating manual for the temperature-validation system.

Validate: To check the device temperature with the aid of a temperature-measuring device. This measuring device forms part of the temperature-validation system and is connected via the serial interface of the Mastercycler.

Calibrate: To align the device temperature with the aid of a temperature-measuring device. This measuring device forms part of the temperature-validation system and is connected via the serial interface of the Mastercycler.

## 5.5 Lid

The "Lid" menu in the main menu can be used to determine the temperature of the heated lid and to switch the heated lid on or off (e.g. to preheat the lid).

Main-Menu Start	L	id Tem	р
FILES			
OPTIONS	LidTemp:		105/55°C
Lid	Heater		is Off
Incubate			
STANDARD			
<i>−−</i> /22.0°			

LidTemp: Nominal/actual temperature.To enter the temperature for the heated lid. The lid heating switches off when a temperature value of 0 °C is entered.

Heater is On: Lid heating is on.

Heater is Off: Lid heating is off.

When sensitive PCRs are carried out, it may be important to delay the start until the lid temperature has prevented condensation. This can be achieved by programming the "WAIT" setting, which means that the program does not start until the lid reaches the specified temperature.

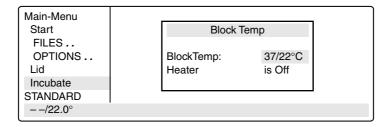
In addition, the lid can also be preheated. After the device has been switched on, the "Lid" menu is selected, the desired lid temperature is entered and "Heater is On" is selected by pressing the selected by pressing the selected.

When a program starts, it is the program settings which have priority.

When a program is running, the lid heating cannot be switched on or off.

## 5.6 Incubate

The "Incubate" menu in the main menu is used to determine the block temperature and to switch on/off the temperature control of the block. (e.g. for incubation experiments or to preheat the block). The temperature of the heated lid is aligned to that of the block.



BlockTemp:Nominal/actual temperature. To enter the temperature for the thermoblock.<br/>It is possible to enter values from +4 °C to 99 °C. It is not possible to select a gradient.

Heater is On: Temperature control of the block is on.

Heater is Off: Temperature control of the block is off.

Another option is to reduce the program run time if the thermoblock and the heated lid have reached the required temperature before the program starts.

To bring the thermoblock and the heated lid to the desired temperature, select the "Incubate" menu after the device has been switched on, enter the desired block temperature and select "Heater is On" using the sel key.

If "Incubate" is running, the "Lid" menu cannot be selected.

When a program starts, it is the program settings which have priority.

When a program is running, the temperature control of the block cannot be switched on or off.

To interrupt: If "Heater is On" is in the display, set the device to "Heater is Off" using the sel key and confirm by pressing enter.

*Caution:* If the "Incubate" menu has been selected, the thermoblock and the heated lid may be hot when the tubes are inserted, which means that there is a high burns risk for the user.

# Operation

## 6.1 Preparation

#### 6.1.1 Loading the samples

The thermoblock can be loaded with three different types of tubes without needing to be exchanged. It can accommodate 96 PCR test tubes of 0.2 ml or 77 thin-walled PCR test tubes of 0.5 ml (special tube from Eppendorf) or 1 microtiter plate (8x12).

To achieve optimal temperature transition, only sample tubes and microtiter plates with a V-shaped base should be used (see "Ordering information"). Test tubes must fit tightly into the thermoblock and should not wobble or move in any way. Tubes which are not sufficiently temperature-stable (up to approx. 120 °C) should not be used.

In general, this also applies to foils used to seal microtiter plates. However, due to the temperature-controlled lid heating, it is also possible to use foils which would melt above 100 °C. In this case, the lid heating can be set, for example, to 95 °C.

The thermoblock has 96 narrow positions for 0.2 ml test tubes and microtiter plates and 77 wide positions for 0.5 ml test tubes. To enable the maximum possible number of 0.5 ml test tubes to be inserted into the thermoblock, the tubes must be positioned in rows. In addition, in order to ensure that the heated lid is closed evenly, it is strongly recommended to position a tube in each corner of the thermoblock. Loading should be carried out as follows:

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	96 x 0.2 ml PCR test tubes or microtiter plates (8x12) (PCR plate 96)
1       2       3       4       5       6       7       8       9       10       11       12         A       - <td>77 x 0.5 ml thin-walled PCR (special tube from Eppendo</td>	77 x 0.5 ml thin-walled PCR (special tube from Eppendo
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	39 x 0.5 ml Eppendorf Safe 0.5 ml test tubes with wide l (e.g. 0.5 ml Eppendorf micro should be loaded in a stagg

-walled PCR test tubes om Eppendorf)

pendorf Safe-Lock micro test tubes

es with wide lid pendorf micro test tubes) ed in a staggered way

in situ reactions require the in situ Adapter. With the aid of the "pegs" on the base, the metal carrier for a maximum of four glass slides is inserted into the 0.5 ml positions of the thermoblock.

# 6 Operation

#### 6.1.2 Sample volume

Temperature control can be set to thermoblock or to sample. The device can adapt to the actual sample quantity and test tube type via the command "CNTRL /TUBE". For maximum flexibility, the program will prompt for tube sizes and volumes when it is started.

The following sample quantities should be used: 0.2 ml test tubes or "plate": not larger than 50  $\mu$ l 0.5 ml or 0.5 ml thin-walled test tubes: not larger than 100  $\mu$ l

When "in situ" is selected, the sample quantity is not taken into consideration.

## 6.1.3 Heated lid

After the samples have been loaded, the heated lid is closed over the thermoblock and locked using the locking lever. The locking lever is moved to the right until the correct tube height has been reached. The symbols indicate the sealing positions for tubes and plates in accordance with the height for a capacity of 0.5 ml, 0.2 ml or 0.1 ml. When plates are loaded, the position "0.2 ml tube" or "Plate" must be selected accordingly.

*Note:* Some types of PCR plates lock into position at the 0.2 ml tube level. Do not force the locking lever when this happens.

Since the heated lid prevents the liquid from condensing in the upper part of the tube, it is not necessary to use an oil layer.

*Note:* Different tube types should not be loaded together as they cannot be sealed correctly by the heated lid. If it is absolutely necessary to process different tube types simultaneously in the thermoblock, an oil layer is necessary for smaller tubes.

The lid temperature (up to 110  $^{\circ}$ C) is controlled automatically by the program. The user can decide whether the program should start immediately (NOWAIT) or whether it should start after a preset lid temperature has been attained (WAIT). If the program has to start quickly, the heated lid can be preheated using the "Lid" menu.

All tubes are easily accessible during the temperature-control procedure since the lid can be opened at any time.

*Caution:* To avoid the risk of burns (110 °C) caused by touching the inner surface of the lid or the thermoblock, do not open the lid while the program is running.

Depending on the processing temperatures used, test tubes and microtiter plates may also be extremely hot.

During *in situ* reactions, the heated lid is not temperature-controlled and remains open at all times.

### 6.1.4 Temperature-control of the block

To carry out incubation experiments or to speed up the start of a method, it is possible to temperature-control the thermoblock with the heated lid. The "Incubate" menu is selected in the main menu, a block temperature is entered and "Heater" is set to "On".

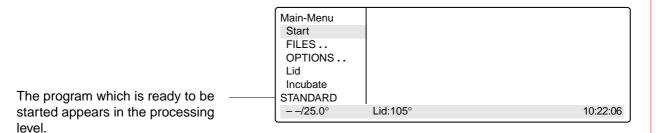
*Caution:* Since the thermoblock and the heated lid can become very hot, there is a burns risk for the user when test tubes and microtiter plates are inserted.

## 6.2 Switching on the device

 Switch on the device using the main power switch on the rear of the device.

The current software version appears briefly in the display. The main menu then appears.

Main-Menu Start FILES OPTIONS Lid Incubate		*** MASTERCYCLER *** gradient 5331 eppendorf Version *.**.**	
STANDARD /25.0°	Lid:105°		10:22:06



## 6.3 Starting a program

- The program in the processing level can be started immediately by pressing the  $\binom{\text{Start}}{\text{Stop}}$  key.

To start another program,

- press Enter

A list of the programs appears in the display.

Select the program name using the
 ♥ or ▲ key (the program name appears with a dark background).

The procedure for starting programs which are stored on a personal card is described in Sec. 9.3.

- Press Enter

The program starts immediately after a "Testing Program".

Main-Menu		Run a Program:	
Start	STANDARD		
FILES	TEST1		
OPTIONS	TEST2		
Lid			
Incubate			
STANDARD			
<i>−−</i> /25.0°	Lid:105°		10:22:06

Main-Menu		Run a Program:
Start	1	T=95.0° 0:00:05
FILES		
OPTIONS		
Lid		
Incubate		
Running/STANDARD		Cyc:4
95.0/94.7°	Lid:105°	10:22:06

# 6 Operation

If the program contains the "CNTRL/TUBE" command (temperature control set to sample), it is necessary to enter the tube type and the filling volume after the program has started.

- Select the tube type: 0.2 ml, 0.5 ml (e.g. Safe-Lock), 0.5 ml thin (thinwalled tubes), plate (microtiter plate) or *in situ* using the sel key and confirm by pressing <u>Enter</u>.
- Enter the filling volume and confirm by pressing Enter.

Tube type 0.2 ml/plate: 5 to 50  $\mu$ l Tube type 0.5 ml/thin: 5 to 100  $\mu$ l *in situ:* no entry necessary

If no entries are made for "Tube" and "Fill.Vol.", the program will not run. "EnterTube" appears in the main menu instead of the program name and a long acoustic signal is emitted until the entry is made.

After the start of the program and while it is running, the name of the program appears in the main menu in alternation with "Running".

A flashing temperature display indicates that the nominal temperature has not been attained and that heating/cooling is currently taking place.

 The display of a program can be exited at any time (e.g. to program in the "Edit" processing level) by pressing [Exit].

Main-Menu		Run a Pro	gram:	
Start		CNTRL	TUBE	
FILES	Tube: 0.2 ml			
OPTIONS				
Lid				
Incubate				
Enter Tube				
22.0/25.0°	Lid:105°			10:22:06

Main-Menu		Run a Pro	ogram:	
Start		CNTRL	TUBE	
FILES OPTIONS Lid Incubate	Fill.Vol.: 20 μl			
STANDARD				
22.0/25.0°	Lid:105°			10:22:06

Main-Menu	Run a Program:			
Start	1	T=95.0°	0:00:05	
FILES				
OPTIONS				
Lid				
Incubate				
- Running/STANDA	RD		Cyc:4	
95.0/97.0°	Lid:105°		10:22:06	
2	3 4	4	56	

- 1 Program flashes in alternation with "Running"
- 2 Nominal/actual block temperature
- 3 Lid temperature (only when lid is switched on)
- 4 Program line number, program command Example: Temperature command (T) with nominal temperature and cycle length
- 5 Number of completed cycles
- 6 Time

## 6.4 Displaying the running time of a program

While a program is running, the approximate run time and the expected end time of the program can be shown in the display.

- Press (opt).

The key is effective only when a program run appears in the display, not in the main menu, with other menus or sub-menus.

Main-Menu		Run a Prog	ram:	
Start	Run Time	: 1	:03	
FILES	Finish Tin	ne:		
OPTIONS	17/Jun/ <sup>-</sup>	1997 15:06		
Lid				
Incubate				
Running/STANDA	RD			
22.0/25.0°	Lid:105°		14:	02:30
	_		_	
	Progra	am end	Currer	nt time
Run time in hours:minutes				

## 6.5 Interrupting a program

A program can be aborted, paused or continued at any point by pressing (Start Stop)

Using the  $(s_{el})$  key, select between:

Stop	To abort the program
Pause	To interrupt the program
Run	To return to the display of
	the program sequence

- Using the (sel) key, move to "Pause".

The program is interrupted, time counting stops and the last nominal temperature is retained.

The name of the interrupted program appears in the main menu in alternation with "Paused" and the "Start" menu changes to the display "Stop".

Main-Menu		Run a Program:		
Start	STANDARD	running		
FILES	Program:	STOP		
OPTIONS				
Lid				
Incubate				
Running/STANDARD				
95.0/94.5°	Lid:105°		10:22:06	

Main-Menu		Run a Program:	
STOP !	STANDARD	STANDARD running	
FILES OPTIONS Lid Incubate	Program:	PAUSE	
Paused/STANDARD			
95.0/94.5°	Lid:105°		10:22:06

# 6 Operation

## 6.6 Continuing an interrupted program

The name of the program appears in the display in alternation with "Paused"

- Press (Start Main-Menu Run a Program: STOP ! STANDARD PAUSED Using the (sel) key, select between: FILES ... Program: PAUSE Stop To abort the program OPTIONS ... To interrupt the program Pause Lid Resume To continue the program Incubate Paused/STANDARD - Using the  $(s_{el})$  key, move to "Resume". 95.0/94.5° Lid:105° 10:22:06
- Confirm "Program:RESUME" by pressing Enter; the program is continued.

Main-Menu		Run a Program:	
STOP !	STANDARD	) PAUSED	
FILES OPTIONS	Program:	RESUME	
Lid			
Incubate			
Paused/STANDAF	RD .		
95.0/94.5°	Lid:105°		10:22:06

## 6.7 Aborting a program

- Press (Start Stop)
- Confirm "Program:STOP" by pressing Enter .

Main-Menu		Run a Program:	
STOP !	STANDARI	D running	
FILES	Program:	STOP	
OPTIONS			
Lid			
Incubate			
Paused/STANDAR	D		
95.0/94.5°	Lid:105°		10:22:06

## 6.8 Switching off the device

After a program has been completed, the following appears in the display:

Main-Menu		Run a Program:	
Start	End of program	rtan a riogram.	
FILES OPTIONS			
Lid			
Incubate			
STANDARD			
22.0/25.0°	Lid:105°		14:02:30

If a new program has to be started, exit the old program by pressing  $(E_{xit})$ .

To switch off the device, move the main power switch on the rear of the device to •.

## 7.1 Command description

A program contains two basic commands for controlling the temperature of the thermoblock and of the heated lid as well as six different commands for programming.

A program can contain up to 40 program lines. The commands may be repeated as desired.

Digits or letters may be entered or selections are made using the (sel) key.

When digits are entered, the message "Value out of range" appears when values are entered which are not within the given specifications. The permitted limit value is then entered automatically.

		'D	
FILES Edit Load Standard New Delete UNNAMED	Edit UNNAME	D CNTRL LID=0° NOWAIT	BLOCK AUTO
	1	T=****° +0.0° R=3.0°/s G=0.0°	**:**:** +0:00 +0.0°/s
	2	Hold ****°	ENTER
	3	PAUSE PRESS	ENTER
	4	GOTO***	REP***
	5	SOUND**	
	6	LINK*******	
		end	
<i>−−</i> /22.0°	10:22:06		

#### 7.1.1 CNTRL

With this command is determined the type of temperature control for the block.

**BLOCK** The temperature is measured on the thermoblock and the nominal temperature is then set.

**TUBE** The software adapts the temperature of the thermoblock to the temperature of the tubes and the sample quantity. For this reason, the tube type and the filling volume must be entered immediately after the start of the program. No further entries are necessary when *in situ* is selected.

Tube type 0.2 ml or plate:	5 to 50 $\mu$ l filling volume
0.5 ml or 0.5 ml thin:	5 to 100 $\mu l$ filling volume

# 7 Programming

## 7.1.2 Lid

To determine the temperature of the heated lid. If 0 °C is selected, the heated lid remains switched off.

Permitted values	0 to 110 °C
Entry increments	1 °C

In addition to the entry for the temperature of the heated lid, it is also possible to determine the behavior of the lid temperature at the start and at the end of a program:

#### At the start

NOWAIT	The program is started, independent of the lid temperature.
-	- F - <b>3</b>

WAITThe program is not started until the programmed lid temperature has been attained.Note.To accelerate the start of the program, the lid may be preheated using the "Lid" menu.

#### At the end

- *FIX* The lid temperature does not depend on the block temperature. The lid heating is switched off at the end of the program.
- *AUTO* The lid temperature depends on the block temperature. If 22 °C is maintained for longer than five minutes, the lid heating switches off; this is also applicable in the case of a Hold command at temperatures < 22 °C.

The six commands for programming can be called up directly using the allotted number.

## 7.1.3 T Selection also possible using 1

To enter the temperature and the cycle time as well as the accompanying specific options.

The options are entered by positioning the cursor on the program line number and pressing the opti key. The options may be entered for every temperature command.

T=****°	** ** **	Nominal temperature, cycle time
+ 0.0 °	+ 0:00	Temperature increment, time increment
R = 3 °/s	<i>+ 0.0 %</i> s	Ramp, ramp increment
G = 0.0		Gradient (for Mastercycler <sup>®</sup> gradient only)

 $T = ****^{\circ}$  The nominal temperature is entered in degrees Celsius (°C).

Permitted values4 to 99.0 °CEntry increments0.1 °C

\*\*:\*\*:\*\* The cycle time is entered in "hours:minutes:seconds". Permitted values 0:00:01 to 9:59:59 Entry increments 1 s

# 7 Programming

±0.0°	Temperature increment (± °C): For ea value entered here.	ch cycle, the temperature is increased or decreased by the
		not exceed 99 °C. For example, with a temperature he starting temperature cannot be greater than 96.5 °C. en the plus or minus sign.
	Permitted values Entry increments	0.0 to 10.0 °C 0.1 °C
	Entry for temperature increase Entry for temperature decrease	"+" ""
± 0:00	Time increment $(\pm s)$ : For each cycle, (max. 1 minute) entered here.	the time is extended or reduced by the value
	Entry for time extension Entry for time reduction	"+" ""
	Permitted values Entry increments	0:00 to 1:00 1 s
R = 3 °/s	value entered, the more rapidly the he	ed of the heating or cooling curve of a cycler. The higher the eating or cooling takes place. Most reactions require rapid lue. For processes in which heating or cooling should take entered for the ramp.
	Permitted values Entry increments	0.3 to 3 K/s 0.1 K/s
± 0.0 °/s	Please note that the ramp cannot exc	e, the ramp is raised or lowered by the value entered here. eeed 3 K/s, i.e. with an initial ramp entry of 0.3 and a ramp cles is possible. First enter the numerical value and then
	Permitted values Entry increments	0.0 to 1.0 K/s 0.1 K/s
	Entry for increasing the ramp speed Entry for decreasing the ramp speed	"+" "_"
<i>G = 0.0</i> °	The nominal temperature set at "T" is left-hand side and increases gradually means that a temperature difference Sec. 5.4.3). The temperature distribut 0.2 ml test tubes, a maximum of 12 d set when 0.5 ml test tubes are used.	e, each column of the block is set to a different temperature. in the center of the block. The lowest temperature is on the y up to the highest temperature on the right-hand side. This of up to 20 °C across the block can be generated (see ion can be viewed in the OPTIONS/Gradient program. With ifferent temperatures may be set whereas only 11 can be not exceed 99 °C, i.e. if a gradient of +10 °C is built up, the
	Permitted values Entry increments	± 0.0 to ± 10.0 °C 0.1 °C
	The gradient has no effect when the	

## 7.1.4 HOLD Selection also possible using 2

The program holds the temperature at the value which was entered. The program is continued or ended by pressing  $\boxed{Enter}$ .

*Note:* A program should not begin with a HOLD command and a temperature < 22 °C because the temperature-control for the lid will not start. If, for example, 4 °C is required at the beginning of a program, HOLD should be connected to the main program via a LINK command (see Sec. 7.6, "Examples of programming").

#### HOLD XX ENTER

Permitted values	4 to 99 °C
Entry increments	0.1 °C

### 7.1.5 PAUSE Selection also possible using 3

The program is interrupted and the temperature remains at the value for the last temperature command. The program is continued by pressing *Enter*.

A user-defined text may be entered instead of the preset text "PRESS ENTER".

PAUSE PRESS ENTER

Text length 0 to	11 characters
------------------	---------------

### 7.1.6 GOTO Selection also possible using 4

To enter data for cycle repeats. It is possible to branch to another program line number (GOTO) where the program should be continued. The number of times this should be carried out (REP) is also given.

#### GOTO XXX REP XXX

Permitted values	
Program line number	1 to 40
Repeats	1 to 99

#### 7.1.7 SOUND Selection also possible using 5

To emit an acoustic signal (e.g. for displaying a programmed pause or when a program is ended). **SOUND**\*\*

Permitted repeats 1 to 10 tones

### 7.1.8 LINK Selection also possible using 6

To link up with another program in the internal memory. Via the LINK command, the current program is ended and the selected program is started. With the aid of LINK commands, a maximum of five programs may be run in succession. The CNTRL, Lid, NOWAIT/WAIT, AUTO/FIX commands in the program head are not taken into consideration during the subsequent programs.

It is not possible to branch to programs in the processing level ("Edit") or on the personal card using LINK commands (it is possible from the personal card to programs in the internal memory).

5

#### LINK\*\*\*\*\*\*\*

Permitted number of programs

The name of the program to be called up must be entered (select letters using the sel key). If the name of the program itself is entered, the program goes into an endless loop and can only be cancelled by pressing "Stop".

### 7.1.9 END

END appears automatically as the last line of a program. It switches off the temperature control for the block and for the lid.

## 7.2 Creating a new program

The process for entering program commands is identical for the Mastercycler<sup>®</sup> and the Mastercycler<sup>®</sup> gradient. A gradient can also be programmed with the Mastercycler<sup>®</sup> gradient.

Example for a program se	equence:
Initial denaturation	95 °C for 2 minutes
20 cycles:	
Denaturation	95 °C for 45 seconds
Annealing	61 °C for 45 seconds
	A gradient of $\pm$ 10 °C is programmed (with Mastercycler <sup>®</sup> gradient only)
Elongation	72 °C for 45 seconds
Final elongation	72 °C for 2 minutes
Cooling of samples	22 °C

When the gradient is set at 61 °C  $\pm$  10 °C when 12 x 0.2 ml test tubes are used, the temperature distribution in the individual columns of the thermoclock is as follows:

Tube row	1	2	3	4	5	6	7	8	9	10	11	12
Temperature	51.0	51.3	52.5	54.2	56.5	59.1	61.8	64.5	67.0	69.1	70.6	71.5

When 0.5 ml test tubes are used, the 11 rows of the block have the following temperatures:

Tube row	1	2	3	4	5	6	7	8	9	10	11
Temperature	51.1	51.9	53.3	55.4	57.8	60.5	63.2	65.8	68.1	69.9	71.1

# 7 Programming

## 7.2.1 Programming

- Select the "FILES" menu using the v or key and call up by pressing
- Select the "New" menu using the () or () key and call up by pressing Enter

Main-Menu Start			
FILES			
OPTIONS			
Lid			
Incubate			
STANDARD			
<i>−−</i> /22.0°	10:22:06		

FILES	Edit UNNA	MED		
Edit		CNTRL	BLOCK	
Load		LID=0°		
Standard		NOWAIT	AUTO	
New		end		
Delete				
UNNAMED				
<i>−−</i> /22.0°	10:22:06			

### CNTRL BLOCK

 Using the sel key or confirm by pressing Enter

#### LID=105° NOWAIT AUTO

 Enter the values and press the 1, 0, 5 keys. Change "Nowait" and "Auto" (Sel key) or confirm by pressing Enter.

### Initial denaturation

#### *T=95.0° 0:02:00*

- Select command T (Temperature) using the sel key (it may be necessary to press the key several times) and confirm by pressing <u>Enter</u>.
- Enter the temperature, press the 9, 5 keys and confirm by pressing Enter
- Enter the time (seconds:minutes:hours).

Press the (0), (0), (2) keys and confirm by pressing (Enter).

FILES	Edit UNNAM	/IED	
Edit		CNTRL	BLOCK
Load		LID=105°	
Standard		NOWAIT	AUTO
New		end	
Delete			
UNNAMED			
<i>−−</i> /22.0°	10:22:06		
•			

FILES	Edit UNNA	MED	
Edit		CNTRL	BLOCK
Load		LID=105°	
Standard		NOWAIT	AUTO
New	1	T=****°	** ** **
Delete		end	
UNNAMED			
<i>−−</i> /22.0°	10:22:06		

FILES	Edit UNNA	MED	
Edit Load		CNTRL LID=105°	BLOCK
Standard		NOWAIT	AUTO
New Delete	1	T=95.0° end	** ** **
UNNAMED		ond	
<i>−−</i> /22.0°	10:22:06		

FILES	Edit UNNA	MED	
Edit			BLOCK
Load		LID=105°	
Standard		NOWAIT	AUTO
New	1	T=95.0°	00:02:00
Delete		end	
UNNAMED			
<i>−−</i> /22.0°	10:22:06		

## Denaturation

## *T=95.0° 0:00:45*

Programming: see page 30.

## Annealing

## *T=61.0° 0:00:45*

 Press the opt key for options for the temperature command (temperature- and time increment, ramp, ramp increment, gradient).

The cursor must be on the program line number in front of the temperature command.

- FILES ... Edit UNNAMED Edit Lid 105° Load NOWAIT AUTO Standard 0:02:00 1 T= 95.0° New 2 T= 95.0° 0:00:45 0:00:45 Delete <u>3</u> T= 61.0° UNNAMED + 0.0 +0:00 10:22:06 --/22.0°
- Press the (6), (1) keys and confirm by pressing (Enter)
- Press the 4 5 keys and confirm by pressing Enter
- Press Enter to proceed to Gradient or Elongation.

## Gradient

## Possible with Mastercycler® gradient only. G=10.0°

 Enter the gradient, press the 1, 0 keys and confirm by pressing Enter.

## Elongation

*T=72.0° 0:00:45* Select the command T.

- Press the 7, 2 keys and confirm by pressing Enter.
- Press the 4 5 keys and confirm by pressing Enter

## Cycle

## GOTO 2 REP 19

- Select the GOTO command using the sel key (it may be necessary to press the key several times) Confirm by pressing Enter ).
- Enter the line number for the beginning of the program section which is to be repeated.
   Confirm by pressing Enter
   Enter the number of repeats (REP).
   Confirm by pressing Enter

*Note:* The total number of cycles is REP + 1.

FILES	Edit UNNA	MED		
Edit	1	T= 95.0°	0:02:00	
Load	2	T= 95.0°	0:00:45	
Standard	3	T= 61.0°	0:00:45	
New		+ 0.0	+0:00	
Delete		R= 3.0°/s	+0:0°/s	
UNNAMED		G= 10.0°		
<i>−−</i> /22.0°	10:22:06			

				_
FILES	Edit UNNA	MED		
Edit	2	T= 95.0°	0:00:45	
Load	3	T= 61.0°	0:00:45	
Standard		+ 0.0	+0:00	
New		R= 3.0°/s	+0:0	
Delete		G= 10.0°		
UNNAMED	4	T= 72.0°	00:00:45	
<i>−−</i> /22.0°	10:22:06			

FILES	Edit UNNAMED		
FILES			
Edit	3	T= 61.0°	0:00:45
Load		+ 0.0	+0:00
Standard		R= 3.0°/s	+0:0
New		G= 10.0°	
Delete	4	T= 72.0°	00:00:45
UNNAMED	5	GOTO***	REP***
<i>−−</i> /22.0°	10:22:06		

# 7 Programming

## Final elongation

*T=72.0° 0:02:00* Programming see page 30.

FILES	Edit UNNAMED		
Edit		+ 0.0	+0:00
Load		R= 3.0°/s	+0:0
Standard		G= 10.0°	
New	4	T= 72.0°	00:00:45
Delete	5	GOTO 2	REP 19
UNNAMED	6	T= 72.0°	00:02:00
<i>−−</i> /22.0°	10:22:06		

#### Cooling of samples HOLD 22.0°

OLD 22.0° ENTER

- Select the HOLD command using the sel key (it may be necessary to press the key several times) and confirm by pressing Enter.
- Enter the temperature.
   Confirm by pressing Enter.

- Exit the "New" menu using the
   Exit key.
- To save, press the Enter key; the device asks for the program name.

In the case of a new program, the device suggests the name "UNNAMED".

- Confirm the name by pressing Enter or – if an other name is desired – delete by pressing Del (if the key is held down, the entire name is deleted; if the keys is pressed briefly, individual letters only are deleted).
- Enter the new program name.
   Select letters using the sel key.
   Press the key to move to the next position etc.
   and confirm by pressing Enter.

If the program is to be saved under an existing name, the question appears:

FILES	Edit UNNAMED		
Edit		R= 3.0°/s	+0:0
Load		G= 10.0°	
Standard	4	T= 72.0°	00:00:45
New	5	GOTO 2	REP 19
Delete	6	T= 72.0°	00:02:00
UNNAMED	7	Hold 22.0°	ENTER
<i>−−</i> /22.0°	10:22:06		

FILES	Edit UNNAMED
Edit	UNNAMED not saved
Load	SAVE: YES
Standard	ProgName: UNNAMED
New	
Delete	
UNNAMED	
<i>−−</i> /22.0°	10:22:06

	· · · · · · · · · · · · · · · · · · ·	
FILES	Edit UNNAMED	
Edit	UNNAMED not saved	
Load	SAVE: YES	
Standard	ProgName: Gradient	
New		
Delete		
UNNAMED		
<i>−−</i> /22.0°	10:22:06	

"Overwrite:YES"
 Confirm overwriting by pressing
 Enter

If you do not want to overwrite the existing program, select "NO" using the  $(s_{el})$  key, press (Enter) and enter a new name. Confirm the new name by pressing (Enter).

FILES	Edit UNNAMED
Edit	Testing
Load	Gradient exists
Standard	Overwrite: YES
New	
Delete	
UNNAMED	
<i>−−</i> /22.0°	10:22:06

If a program is not saved ("Save:NO"), it remains in the processing level and can be supplemented or modified by calling up "Edit". Safety checks in other sub-items prevent a program which has not been saved from being accidentally overwritten.

The internal memory can accommodate a maximum of 99 programs. The number possible depends on the length of the programs.

The program in the processing level can be started by pressing the  $\binom{\text{Start}}{\text{Stop}}$  key.

*Note:* The procedure for saving programs on a personal card is described in Sec. 9.3.

# 7.3 Modifying a program

A program which is already in the internal memory or on a personal card can be modified by being loaded into the processing level. The program resident in the "Edit" processing level is thus deleted. If a program which has not yet been saved is in the processing level, it can be saved following a safety check prior to deletion.

Select the "FILES" menu using the
 or key and confirm by
 pressing Enter

Main-Menu Start	
FILES	
OPTIONS	
Lid	
Incubate	
STANDARD	
<i>−−</i> /22.0°	10:22:06

Select the "Load" menu using the
 or key and call up by
 pressing Enter

A list of existing programs appears in the display.

Select the program using the v or
 key and load by pressing <a href="mailto:tenter">tenter</a>

FILES	Load to Editor
Edit	STANDARD
Load	TEST1
Standard	TEST2
New	
Delete	
STANDARD	
<i>−−</i> /22.0°	10:22:06
	)

FILES	Edit STAN	DARD	
Edit	1	T= 94.0°	0:02:00
Load	2	T= 94.0°	0:00:15
Standard	3	T= 44.0°	0:00:15
New	4	T= 72.0°	0:00:30
Delete	5	GOTO 2	REP 24
STANDARD	6	T= 72.0°	0:00:30
<i>−−</i> /22.0°	10:22:06		

# 7 Programming

- Using the (A), (V), (I), (I) keys or Enter, move the flashing cursor onto the parameter which is to be changed.
- Enter data using the numeric keypad or select between settings using the sel key or enter letters using the sel key.

### Inserting a program line

- Move the flashing cursor onto the number of the program line in front of which a new command is to be inserted.
- Press the (Ins) key. A new line is inserted above the cursor.
- Select the wanted command using the sel key.

*Note:* Press the opt key for entering options regarding the temperature command. In this case, the cursor must be positioned on the program line number in front of the temperature command.

### Deleting a program line

- Move the flashing cursor to the program line number.
- Press the (Del) key. The line is deleted.
- After a program has been changed, exit the "Edit" processing level by pressing the Exit key.
- Confirm the safety check "Save:YES" by pressing Enter
- The modified program can be saved using the same name, in which the old program is overwritten.
   Confirm the check "Overwrite:YES" by pressing Enter.

If the program should not be overwritten, and a new program is to be created, answer "Overwrite:" with "NO" (sel and enter keys) and enter a new program name with sel or the numeric keypad.

*Caution:* If the changed program is not stored, there will be two different programs with the same name. The program which has been changed is in the processing level and the program which has not been changed is in the device memory.

The modified program can be started in the processing level  $\binom{\text{Start}}{\text{Stop}}$ 

- After the end of the program "End of Program" appears in the display. Press the Exit key.
- If you now want to save the program which has been changed, select "Load" menu with v and press
   Enter . "Program not saved Save YES" appears in the display.
- Press Enter to save the program.

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# 7.4 Creating programs using an example program

It is possible to program quickly with the aid of the "Standard" example program. A program does not need to be completely rewritten; the sample can be supplemented, modified and then saved under a new name.

- Select the "FILES" menu and call up by pressing Enter.
- Select "Standard" and call up by pressing Enter

The example program is loaded into the processing level. It is structured in the following way:

	CNTRL LID=105°		BLOCK	Temperature control for the block. Sets heated lid to 105 $^{\circ}$ C.
	NOWAIT		AUTO	NOWAIT = Program is started immediately. AUTO = Lid heating is switched off automatically when temperature (22 °C) is maintained for more than 5 minutes.
1	T=94.0°		0:02:00	Maintains 94 °C for 2 min. (initial denaturation)
2	T=94.0°		0:00:15	Maintains 94 °C for 15 secs. (denaturation)
3	T=44.0°		0:00:15	Maintains 44 °C for 15 secs. (annealing)
1	T=72.0°		0:00:30	Maintains 72 °C for 30 secs. (elongation)
5	GOTO 2		REP 24	Repeats 24 times from program step 2 onwards. The total number of cycles is 25.
3	T=72.0°		0:00:30	Maintains 72 °C for 30 secs. (final elongation)
7	HOLD	22.0°	ENTER	Cools down to 22 °C and maintains temperature.
	end			Program end

Although the example program cannot be deleted, individual changes can be made. After changes have been made, the example program must be saved under the name "Standard". To restore the original example program, the "Standard" program in the "Delete" sub-menu must be deleted. When "Standard" is called up, the original example program is reloaded into the processing level.

# 7.5 Deleting a program

- Select the "FILES" menu and call up by pressing Enter
- Select "Delete" and call up by pressing Enter.
   A list of the existing programs appears in the display.

FILES	Delete Program
Edit	TEST 1
Load	TEST 2
Standard	
New	
Delete	
STANDARD	
<i>−−</i> /22.0°	10:22:06

### - Select the program name.

- Confirm by pressing Enter. The program is deleted.

The procedure for deleting programs on a personal card is described in Sec. 9.3.

# 7.6 Examples of programming

The following passage contains several examples of the versatility of the commands and functions of the Mastercyclers.

# 7.6.1 Gradient PCR (for Mastercycler® gradient only)

The Gradient PCR is used to optimize temperatures in a PCR experiment. The gradient may be programmed with a temperature range of up to 20 °C with every temperature command.

The most common application is the determination of the optimal annealing temperature (see example), for which a gradient of, for example,  $\pm$  10 °C is built up. The recommended mean gradient is 5 °C higher than the calculated annealing temperature.

	Gradient PCR		
	Temperature control of tubes	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	<b>94</b> °	2 min
2	Denaturation	<b>94</b> °	15 sec
3	Annealing	<b>60</b> °	15 sec
	Gradient	G = 10°	
4	Elongation	<b>72</b> °	30 sec
5	Number of cycles	go to 2	Rep 29
6	Final elongation	<b>72</b> °	2 min
7	Cooling and storage	Hold 20°	enter
	End	end	

### 7.6.2 Using the "Pause" and "Hold" commands (Example: Hot Start PCR)

The "Pause" command is used to interrupt the program. The most-recently entered temperature is maintained. A text explaining the reason for the interruption may be entered as required. After confirmation by pressing Enter, the program is continued.

The "Hold" command enables all possible temperatures to be maintained until the program is continued by pressing Enter.

In the example of a Hot Start PCR listed below, the program is stopped following initial denaturation in order to add a reaction component. After the final elongation, the block is cooled down to room temperature.

	Hot Start PCR		
	Temperature control of tubes	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	<b>9</b> 4°	2 min
2	Pause for adding substance	Pause	Adding
3	Denaturation	<b>94</b> °	15 sec
4	Annealing	55°	15 sec
5	Elongation	<b>72</b> °	30 sec
6	Number of cycles	go to 3	Rep 29
7	Final elongation	<b>72</b> °	2 min
8	Cooling and storage	Hold 20°	enter
	End	end	

# 7.6.3 The temperature increment (Example: Touch Down PCR)

The temperature increment, which can be programmed as desired with every temperature command, allows the temperature to be reduced or increased by a defined value with every cycle.

Touch Down PCR can be used to increase the specificity of the PCR by changing the the annealing temperature in succession from higher to lower temperatures.

In the example given, the annealing temperature is reduced by 1 °C per cycle for the first 16 cycles, until 50 °C has been attained. At this annealing temperature,14 additional cycles are carried out.

	Touch Down PCR		
	Temperature control of tube	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	<b>9</b> 4°	2 min
2	Denaturation	<b>94</b> °	15 sec
3	Annealing	65°	15 sec
	Temperature increment	<b>-1</b> °	
4	Elongation	<b>72</b> °	30 sec
5	Number of cycles	go to 2	Rep 15
6	Denaturation	<b>94</b> °	15 sec
7	Annealing	<b>50</b> °	15 sec
8	Elongation	<b>72</b> °	30 sec
9	Number of cycles	go to 6	Rep 13
10	Final elongation	<b>72</b> °	2 min
11	Cooling and storage	Hold 20°	enter
	End	end	

### 7.6.4 The time increment (Example: Long PCR)

The time increment, which can be programmed as desired with every temperature command, enables a defined extension of the lag time at a specific temperature for each cycle.

With Long PCR, this function can be used to amplify long fragments for a successive extension of the elongation.

In the example given, the elongation time is extended by 10 seconds for each cycle, which means that, for the 30th cycle, the elongation time is 290 seconds longer than for the first cycle.

	Temperature control of tubes	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	<b>94</b> °	2 min
2	Denaturation	<b>94</b> °	15 sec
3	Elongation	68°	10 min
	Time increment		+0:10 sec
4	Number of cycles	go to 2	Rep 29
5	Final elongation	<b>72</b> °	15 min
6	Cooling and storage	Hold 20°	enter
	End	end	

### 7.6.5 Regulating the temperature-control speed (Example: RAPD-PCR)

Variable heating and cooling rates, which can be programmed as desired with each temperature command, enable the temperature-control speed to be aligned to the temperature.

With the RAPD-PCR process or with PCR with A/T-rich primer/template hybrids, it may be necessary to heat up carefully after annealing.

In the example given, the elongation temperature is attained at a speed of 1 K/s.

	RAPD-PCR		
	Temperature control of tube	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	<b>9</b> 4°	2 min
2	Denaturation	<b>94</b> °	15 sec
3	Annealing	55°	15 sec
4	Elongation	<b>72</b> °	30 sec
	Heating rate	R = 1 °/s	
5	Number of cycles	go to 2	Rep 29
6	Final elongation	<b>72</b> °	2 min
7	Cooling and storage	Hold 20°	enter
	End	end	

Temperature-control speeds, which may be as slow as the user desires, can be selected by means of program loops, with which a gradual change in temperature is obtained via temperature increments.

In the following example, the elongation temperature is raised slowly to 72 °C after annealing with the aid of a program loop.

	RAPD-PCR		
	Temperature control of tube	Control	tube
	Temperature control of lid	lid	105°
		Nowait	auto
1	Initial denaturation	<b>9</b> 4°	2 min
2	Denaturation	<b>94</b> °	15 sec
3	Annealing	55°	15 sec
4	Elongation	56°	7 sec
	Temperature increment	+1°	
	Heating rate	R = 0.3 °/s	
5	Number of cycles	go to 4	Rep 16
6	Number of cycles	go to 2	Rep 29
7	Final elongation	<b>72</b> °	2 min
8	Cooling and storage	Hold 20°	enter
	End	end	

# 7 Programming

# 7.6.6 Sample cooling and subsequent PCR

If sensitive samples should remain cooled until the PCR takes place, the thermoblock must be preheated. This can be carried out in two ways:

- 1. Set the "Incu" menu to 4 °C and "Heater is On".
- Adapt an initial program and connect via a "LINK" command. For this purpose, the temperature of the thermoblock is brought to 4 °C using the first program. After the samples have been inserted into the cold thermoblock, the second program with the PCR protocol is called up via a LINK command.

Programming should take place as follows:

Program 1	e.g. COOL
CNTRL TUBE Lid NOWAIT FIX	The entries in the first program are also valid for the programs which are added via LINK. The lid setting "Fix" must be selected.
1 Hold 4 °C Enter	The thermoblock cools until the processing program (e.g. PCRPROT) is started via the LINK command by pressing Enter.
2. Link PCRPROT end	Link to the second program with the PCR protocol.
Program 2	PCRPROT
CNTRL TUBE	If the program also contains the "Tube control" command, further entries for

first program are valid for all further programs.

tube type and filling volume are no longer necessary; the entries made for the

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It is essential to read the operating manual completely before working with the short instructions.

### Switching on the device

Menu name	Main-Menu Start FILES OPTIONS Lid Incubate STANDARD		
Nominal/actual block temperature	/25.0°	Lid:105°	10:22:06
Lid temperature (only when lid is switched on)			Time

#### Programming

- Select the FILES menu using the ( ) / ( ) keys and press $\_$ Enter $]$ .				
	FILES			
To edit the program in the processing level	Edit			
To load and edit any program	Load			
To load and process the example program ———	Standard			
To create a new program —	New			
To delete any program	Delete			
	STANDARD			
	<i>−−</i> /22.0°		10:22:06	

### Changing the "Standard" example program

- Select "Standard" using the V key and press Enter.
- Using the ( $\blacktriangle$ ), ( $\bigtriangledown$ ), ( $\blacklozenge$ ), keys, move the cursor to or between the entry positions.
- Select the program commands using the (Sel) key, enter the settings or the texts and confirm by pressing Enter

CNTRL	To define temperature regulation	FILES		Edit STANDA		
BLOCK	For the thermoblock	Edit		CNTRL	BLOCK	
TUBE	For different tubes	Load		LID=105°		
Lid	To determine the lid temperature	Standard		NOWAIT	AUTO	
NOWAIT	To start a program immediately	New	1	T=94.0°	0:02:00	
WAIT	Program waits for lid temperature	Delete	2	T=94.0°	0:00:15	
AUTO	Lid switches off at <22 °C	STANDARD	3	T=44.0°	0:00:15	
FIX	and time >5 min. Lid switches off at end of program	<i>−−</i> /22.0°	•			10:22:06

#### Program commands

Т	Temperatures, times, increments, ramp, gradient
HOLD	To maintain temperature entered until program is continued with <i>Enter</i> (4–99 °C)
PAUSE	To interrupt program until program is continued with <i>Enter</i> (0–11 characters)
GOTO/REI	P To repeat program parts and number of cycles (1-40 lines, 1-99 repeats)
SOUND	Acoustic signal (1–10 tones)
LINK	To branch to other programs (1-5 programs)

Enter the options for the temperature command using the opt key. The cursor must be positioned on the program line number.

Т	Temperature (0–99°)
	Time (0:00:01–9.59:59)
	Temperature increment (0.0 – 10.0°, + or –)
	Time increment (0:00–1:00, + or –)
R	Ramp (0.3–3 K/s)
	Ramp increment (0.0–1.0 °/s, + or –)
G	Gradient ( $\pm 0,0 - \pm 10$ °C)
	Gradient (±0,0 – ±10 °C) (for Mastercycler <sup>®</sup> gradient only)

FILES		STANDA	RD	
Edit	3	T=44.0°	0:00:15	
Load	4	T=72.0°	0:00:30	
Standard		+0.0°	+0:00	
New		R= 3.0°/s	+0.0°/s	
Delete	1	G=0.0°		
STANDARD	5	GOTO 2	REP 24	
<i>−−</i> /22.0°				10:22:06

- Confirm entries by pressing Enter
- Complete programming by pressing [ Exit
- Save the program.

# 8 Short instructions

### Saving a program

- Exit the program using the Exit key.
- Answer the question "Save: YES" by pressing Enter
- Define the program name using the (sel) key.
- Confirm the name by pressing Enter

### Adding a program line

- Using the  $(\blacktriangle)/(\bigtriangledown)$  keys, move the cursor to the front of the line. \_
- \_ Press the (Ins) key; a new line is then added above the cursor. Select command required with (sel).

FILES . .

Edit

Load

New

Delete STANDARD

Standard

– –/22.0°

. .

### Deleting a program line

- Using the  $(\blacktriangle)/(\bigtriangledown)$  keys, move the cursor onto the program line number.
- Press the (Del) key; the line is then deleted and the cursor moves to the next line. \_

### System settings

	0	
To temp	perature-control lid an	d block Incubate menu
To preh	eat the lid	Lid menu
	programming	OPTION menu
To print	program sequence	OPTION menu

### Starting a program

- Switch on the devi preheat the lid and the block.
- Select the "Start" menu using the  $(\blacktriangle)/(\blacktriangledown)$  keys and press Enter
- Select the program name using the (A) / (V) keys and press Enter
- With CNTRL TUBE, select the tube type using the  $(s_{el})$  key. Enter the filling volume. 0.2 ml/plate: 5 to 50 µl 0.5 ml/thin: 5 to 100 µl
- Confirm by pressing Enter The program starts immediately.
- To see the program run time, press the  $(o_{pt})$  key.

### Interrupting a program

- Press the  $\binom{\text{Start}}{\text{Stop}}$  key.
- Using the (sel) key, select the "Pause" program.
- Confirm by pressing Enter

### Continuing an interrupted program

- Press the (Start) ) key.
- Using the sel key, select "Resume".
- Confirm by pressing Enter

### Stopping a program

- Press the  $\binom{\text{Start}}{\text{Stop}}$  key.
- Confirm "Stop" by pressing Enter
- Exit the program by pressing Exit

Main-Menu		Run a Program:	
Start	STANDARD	) running	
FILES OPTIONS Lid Incubate STANDARD	Program:	STOP	
95.0/94.5°	Lid:105°		10:22:06

Block temperature 37 °C, Heater is ON

STANDARD not saved

ProgName: STANDARD

SAVE: YES

Edit STANDARD

10:22:06

ence	Lid menu OPTION menu OPTION menu	LidTemp 1 Printout Ec		
ice. If necessa	ry,	Main-Menu Start	STANDARD	Rı

Run a Program: STANDARD FILES .. TEST1 OPTIONS .. TEST2 Lid Incubate STANDARD --/25.0° 10:22:06

Main-Menu		Run a Pr	ogram:	
Start	1	T=95.0°	0:00:05	
FILES				
OPTIONS				
Lid				
Incubate				
Running/STANDARD			Cyc:4	
95.0/94.5°	Lid:105°		10:	:22:06

Main-Menu		Run a Program:	
Start	STANDARD r	unning	
FILES OPTIONS	Program:	PAUSE	
Lid Incubate			
Paused/STANDARD			
95.0/94.5°	Lid:105°		10:22:06

Depending on the length of the program, a maximum of ten programs can be stored on a personal card. When a program is started from a personal card, the program is loaded into a temporary memory (but not into the internal memory or into the processing level) for the duration of the program processing.

Programs can be transferred to other devices of the Mastercycler<sup>®</sup>, Mastercycler<sup>®</sup> gradient and Mastercycler<sup>®</sup> personal using the personal card.

For the "Start", "FILES/Load", "FILES/Edit" and "FILES/Delete" menus, a difference will be made between the memory of the personal card  $\langle MCARD \rangle$  and the internal memory of the Mastercycler<sup>®</sup>  $\langle INTERN \rangle$  only when the personal card has been inserted.

Menus "Start", "FILES/Load" and "FILES/Delete"

Only the programs of a memory appear in the display. The memory which is currently closed appears in the display at the head of a program list as  $\langle INTERN \rangle$  or  $\langle MCARD \rangle$ . The closed memory can be opened by selecting and then confirmed by pressing (Enter). This also closes the previous memory.

# Menu "FILES/Edit" The memory target (Target) must be selected using the selected using the selected before the program name is determined.

# 9.1 Safety precautions

The gold-colored contact area on the personal card must not be damaged, scratched or contaminated in any way. Avoid touching the contact area with your fingers.

Electrostatic charges can destroy stored programs.

# 9.2 Operation

Inserting the card

- With the arrow facing forwards and the gold-colored contact area facing upwards, insert the card into the slot under the control panel.
- Insert the card up to the stop. It clicks into place automatically.

### Removing the card

- Insert the card up to the stop.
- The card is pushed out by spring pressure and can then be removed.

### Formatting the card

A new card must be formatted. The request for formatting appears automatically in the display only when it is necessary. The request for formatting is also made if the card has been erroneously inscribed.

 "Memory card not valid" Confirm "Format: Yes" by pressing Enter.

The nominal/actual steps appear in the bottom line of the display.

# 9 Personal card

# 9.3 Processing programs

### Starting a program from a card

- Select the "Start" menu item and call up by pressing Enter.
- Select (MCARD) and call up by pressing <a>Enter</a>
   If the memory name (INTERN) appears in the display instead of (MCARD), this means that the program memory of the personal card has already been opened.
- Select a program and call up by pressing Enter.

*Note:* For starting purposes, programs are not loaded into the internal memory or into the "Edit" processing level. After the program has ended, the program which has been started by the card is no longer available on the device.

### Deleting a program from a card

- Select the "FILES/Delete" menu and call up by pressing
- Select (MCARD) and call up by pressing Enter.
   If the memory name (INTERN) appears in the display instead of (MCARD), this means that the program memory of the personal card has already been opened.
- Select a program and delete by pressing Enter

### Loading a program from a card into the processing level

- Select the "FILES/Load" menu and call up by pressing Enter.
- Select (MCARD) and call up by pressing <a href="mailto:Enter">Enter</a>. If the memory name (INTERN) appears in the display instead of (MCARD), this means that the program memory of the personal card has already been opened.
- Select a program and load by pressing Enter

### Saving a program on a card

- After a program has been compiled or modified, exit the processing level by pressing Exit.
- Confirm the inquiry "Save: YES" by pressing Enter
- For the inquiry "Target:INTERN", select (MCARD) using the sel key and confirm by pressing Enter
- Enter the program name (max. 8 characters) and confirm by pressing Enter

Note: The program can be saved under the same name as in the memory of the Mastercycler.

### Programs with LINK commands

A program cannot be started from a personal card if it contains program branching – LINK commands – to other programs on the personal card. It is possible to start the program if branching to another program in the internal memory of the devices is available.

A program from the internal memory cannot call up a program from the personal card using a LINK command.

# 10.1 Printer / PC connection

### Printer connection

The printer connection socket (25 pins, Centronics parallel interface, PC compatible) is located below the PC connection socket on the right-hand side of the device (see Sec. 4.1, Fig. 2.6).

Connect the Mastercycler<sup>®</sup> or Mastercycler<sup>®</sup> gradient and printer via a commercially available PC printer cable. The cyclers and printer must be switched off during installation. If the printer is not used frequently, the printer cable should be disconnected from the cycler port and only be installed when needed.

The parameters for the printer are set in the "OPTIONS/Printer" menu.

 Print programming
 Printout Editor: NO

 Printing is switched off.
 Printout Editor: YES

 The program in the "Edit" menu is printed out.
 Select "YES" using the Sel key and confirm by pressing Enter.

 "Print program protocol
 Print Protocol: OFF

 The program sequence is not printed out.

 Print Protocol: ON

The program sequence is printed out.

Example of a program printout:

ej Vi Ti D	TER CYCLER g opendorf ersion x.xx.xx me: 13:41:02 ate: 14/Apr/1999 * * * * * * * * * * * *		
LISTIN Program: Created: Step:	G STANDARD 13/Apr/1999	11:46	Program name Date last stored
CNT	RL BLOCK		
	105C WAIT AUTO		
	94.0C 0:02:00		
	-0.0C + 0:00 3.0C/s +0.0C/s 0.0C		
	4.0C 0:00:15		
	-0.0C + 0:00 3.0C/s +0.0C/s		
G = 0	0.0C		
· · · ·			
7 HOL	D 22.0C ENTER		
ENDOFLISTING			
<u> </u>			

### Example of a program protocol:

Prior to the start, the program is printed (see page 46) and during the program sequence, all temperatures and times are printed in a protocol.

Program started at	_	Date and time of start of program
Initial Blocktemp	_	Block temperature at start
Initial Lidtemp	_	Lid temperature at start
Run Time	_	Estimated run time
Finish Time	_	Estimated time for end of program
Count	_	Count of program steps
Cycle	_	Count of cycles
Step	_	Program step no. from programming
Command	_	Command type and programmed values
Time	_	Time at end of a command
Completion Time	_	Time at end of program

Program Initial Blo Initial Lid Run Time Finish Tir	temp: e:	D started	at Date: 24.7 C 25 C 1:26 15/Apr/1999	15/Apr/1999 9:22	7:56:12
Count	Cycle	Step	Command		Time
			Tube:0.2 ml	Fill.Vol.:	20 µl
			CNTRL	TUBE	7:56:21
			LID=105C NOWAIT	AUTO	7:56:22
1	1	1	T=94.0C +0.0C R=3.0C/s G=0.0C	0:02:00 + 0:00 +0.0C/s	7:59:05
2	1	2	T=94.0C +0.0C R=3.0C/s G=0.0C	0:00:15 + 0:00 +0.0C/s	7:59:21
•					
Completion Time:			15/Apr/1999	9:24:55	
END C	)F PRO	TOCOL			

### PC connection

The 9 pin PC connection socket (serial interface RS 232) is located above the printer port on the right-hand side of the device (see Sec. 4.1, Fig. 2.5).

The parameters for the computer are set in the "OPTIONS/GENERAL/Remote" menu.

Selection of transfer rate: Baud rate 19,200, 9,600, 4,800, 2,400 or 1,200

Detailed information about PC connection and the connecting cable required is available on request.

The computer to be connected must correspond to the EN 60950 or UL 1950 regulations.

A special operating manual is available for serial communication. Further information is available on request.

# 10.2 Program transfer

If programs are stored on a personal card, they can be transferred via the card to other Mastercycler<sup>®</sup>, Mastercycler<sup>®</sup> gradient and Mastercycler<sup>®</sup> personal devices. Programs created on a Mastercycler<sup>®</sup> personal can also be transferred to a Mastercycler<sup>®</sup> gradient. The Mastercycler<sup>®</sup> gradient has an additional function, whereby a Gradient function is added to the temperature command, i.e. a temperature gradient can be built up in the thermoblock. This function cannot be used when programs are transferred to the Mastercycler<sup>®</sup> and Mastercycler<sup>®</sup> personal.

The Mastercycler<sup>®</sup> / Mastercycler<sup>®</sup> gradient can be cleaned using water or a mild laboratory cleaning agent. The device should not come into contact with organic solvents or aggressive solutions. Ensure that no liquid enters the device. For safety reasons, the device must be switched off and disconnected from the power supply before cleaning begins.

The electrical safety fuses are located between the main power switch and the main power plug at the rear of the device. They can be removed by sliding them up one catch. Before the fuses are replaced, the device must be switched off and disconnected from the mains supply. Only fuses with the correct voltage values may be used (information on the fuse type can be found at the rear of the device).

The device may only be opened by qualified service personnel. The warranty will not be honored in the event of damage caused by unauthorized servicing.

Programming errors or errors regarding the handling of the personal card can be eliminated after the cause of the error has been established.

Technical errors can be caused by interference (e.g. power failure or power fluctuations). In some cases, it is possible to eliminate the error by switching off the device for a short period. Wait ten seconds before switching on the device again. If the error recurs, contact a service technician.

# 12.1 Error messages

Error message	Cause	Solution
BLOCK ChkSumErr	Technical defect.	Contact SERVICE.
<b>B</b> LOCK TOO HOT!	Defective regulation or defective electronics.	Contact SERVICE.
<b>C</b> ard changed	Card removed immediately before being inscribed.	Insert card correctly and repeat procedure.
<b>C</b> ard content not o.k.	Content of card is defective.	Card must be formatted. Automatic procedure when program is saved.
<b>C</b> ard not in slot	Card removed during saving process.	Insert card correctly and repeat procedure.
<b>E</b> nter Tube	Not all entries have been made for a program which has been started.	Enter tube type and sample volume.
<i>E</i> rr GOTO (1)	Branching to a program line number is impossible because this program line number is in a program area which has already been selected for repeating using another GOTO command.	Check GOTO command and modify or delete if necessary. Program line number for additional GOTO command must not be in a section which is already addressed by a GOTO command.
<b>E</b> rr GOTO (2)	More than three GOTO commands used for one program range.	Three GOTO commands can be used in one program range when the areas which have been addressed overlay each other completely, i.e. the program area of the second command is greater than that of the first command. The third GOTO command must cover an even greater area than that of the second command.
<b>E</b> rr Invalid	Invalid command. Parameter has not been entered.	Enter command completely or delete command.

# 12 Troubleshooting

Error message	Cause	Solution
<b>E</b> rr Link (1)  Line	LINK command cannot be executed. Program not found. Program name and program line of defective LINK command appear in the display.	Only programs from the internal memory can be called up by the LINF command. Programs on a personal card and the program in the processing level ("Edit") cannot be called up by the LINK command.
<b>E</b> rr Link (2)  Line	Too many programs are connected by LINK commands. Program name and program line of defective LINK command appear in the display.	A maximum of five programs, i.e. fou LINK commands, can be processed in succession.
<b>E</b> rr No Program	No executable program available in memory.	Load program from memory or personal card, or define a program in "Edit" menu and restart.
<b>F</b> AN ERROR!	Defective internal fan.	Switch off device. Contact SERVICE.
<b>F</b> AT Full	Number of programs which can be administrated has been exceeded. Internal memory: 99 programs personal card: 10 programs	Delete programs which are not required.
Internal EE-Err	Technical defect.	Contact SERVICE.
<i>L</i> EFT BLOCK DEFEKTIV!	Defective regulator or electronics on left-hand block.	Switch off device. Contact SERVICE.
LID TOO HOT!	Defective electronics.	Contact SERVICE.
<i>L</i> ink not found	Program branching is not possible because the program which was supposed to be called up has been deleted.	Check the LINK command. Enter the missing program.
<i>L</i> ink Error	Program branching is not possible because the program which was supposed to be called up has been deleted.	Check the LINK command. Enter the missing program.
MCard Error	The memory of the personal card is defective.	Evaluate further messages.
<i>M</i> emory corrupted	The internal memory is defective.	The internal memory must be deleted. This deletes all programs.

Error message	Cause	Solution
<i>M</i> emory Error	The internal memory for programs is defective.	Evaluate further messages.
<i>M</i> emory full!	Memory is full.	Before storing, delete programs which are not required.
<b>M</b> emoryCard (Blank)	Card has incorrect logical identification and/or is blank.	Card must be formatted. Automatic procedure when program is saved.
<i>M</i> emoryCard Defective!	Card is defective.	Use a new card.
<b>M</b> emoryCard HdChksumErr	Error in check sum.	Card must be formatted. Automatic procedure when program is saved.
<b>M</b> emoryCard ManIDErr	Incorrect identification.	Card must be formatted. Automatic procedure when program is saved.
<i>M</i> emoryCard MemOverErr!	Incorrect memory address.	Card must be formatted. Automatic procedure when program is saved.
<b>M</b> emoryCard MemRangeErr	Error in memory range.	Card must be formatted. Automatic procedure when program is saved.
<i>M</i> emoryCard MemSizeErr	Incorrect memory size.	Card must be formatted. Automatic procedure when program is saved.
<b>M</b> emoryCard MemUnderErr!	Incorrect memory address.	Card must be formatted. Automatic procedure when program is saved.
<b>M</b> emoryCard Missing!	No card has been inserted.	Insert card.
<i>M</i> emoryCard Not Valid	Card is invalid.	<ul> <li>Insert card correctly (check that card is facing in the correct direction).</li> </ul>
		<ul> <li>Card should be formatted before saving begins.</li> </ul>

# 12 Troubleshooting

Error message	Cause	Solution
<i>M</i> emoryCard Read Err!	Card cannot be read.	Insert card correctly (check that card is facing in the correct direction).
<i>M</i> emoryCard Read-Only!	Card is marked as write-protected.	Card must be formatted. Automatic procedure when program is saved.
<i>M</i> emoryCard Write Err!	Writing error on card.	<ul> <li>Insert card correctly (check that card is facing in correct direction).</li> </ul>
		<ul> <li>Do not touch/move card during saving.</li> </ul>
MID BLOCK DEFEKTIV!	Defective regulator or electronics on middle block.	Switch off device. Contact SERVICE.
<b>N</b> ame Invalid!	Program name contains an invalid character.	Enter program name again.
<b>P</b> OWER ERROR!	Voltage error or defective fuse.	Contact SERVICE.
<b>P</b> rinter: OFF LINE	Printer is not ready to receive.	Select the "ON LINE" printer setting.
<b>P</b> rinter: no response	No printer is available or the printer is not switched on.	<ul> <li>Select "OFF" in the menu OPTION/Printer/Print Protocol.</li> </ul>
		<ul> <li>Switch on the printer.</li> </ul>
<b>P</b> rogram is running!	A program is being processed.	Lid temperature cannot be modified at the moment.
<b>P</b> rogram too large!	Program is too large.	Max. 40 program lines possible.
<b>R</b> estarting Program!	A power failure occurs or the device is switched off briefly when a program is running.	The program continues automatically
<b>R</b> IGHT BLOCK DEFEKTIV!	Defective regulator or electronics on right-hand block.	Switch off device. Contact SERVICE.
<b>S</b> LOWING DOWN!	The heating rate or cooling rate has slowed down.	If error recurs, contact SERVICE.
<b>S</b> teps free	Display of free memory spaces is < 100 steps. 1 step = 1 program line.	Delete programs which are not required or store them on the personal card.

Error message	Cause	Solution
<b>7</b> Sensor_Err	<ul> <li>Permitted operating conditions have not been maintained.</li> </ul>	<ul> <li>Operate device at permitted temperature and humidity only</li> </ul>
	<ul> <li>Technical defect.</li> </ul>	<ul> <li>Contact SERVICE.</li> </ul>
<i>V</i> alue out of range!	Value is not within the permitted value range.	The closest permitted value is entered automatically.
<b>W</b> arn Ramp Max  Line	The ramp is limited to 3 K/s.	Check entries for ramp and ramp increment, and change entries if necessary.
<b>W</b> arn Ramp Min  Line	The ramp is limited to 0.3 K/s.	Check entries for ramp and ramp increment, and change entries if necessary.
<b>W</b> arn Temp Max	Temperature limits of 99 °C exceeded. If program is started, temperature is limited to 99 °C.	Check temperature commands and reduce value of temperature increment so that only values below 99 °C can be reached.
<b>W</b> arn Temp Min	Temperature limit of 4 °C not reached. If program is started, temperature is limited to 4 °C.	Check temperature commands and modify values of temperature increment so that only values above 4 °C can be reached.
<b>W</b> arn Time Max	Cycle time of 9:59:59 exceeded. If program is started, cycle time is limited to 9:59:59.	Check temperature commands and modify values of time increment so that only values below 9:59:59 can be reached.
<b>W</b> arn Time Min	Cycle time of 0:00:01 not reached. If program is started, cycle time is limited to 0:00:01.	Check temperature commands and modify values of time increment so that only values above 0:00:01 can be reached.

# 13 Technical data

Mastercycler <sup>®</sup> / Mastercycler <sup>®</sup> gradient	
Sample capacity:	1 PCR plate 96 or 96  0.2 ml PCR tubes or
	77 0.5 ml PCR tubes, thin-walled
Temperature range:	4 to 99 °C
Temperature uniformity across the block:	20 °C to 72 °C ± 0.6 K 95 °C ± 1.0 K
Block homogeneity ( $\overline{S}_{95}$ ):	20 °C to 72 °C $\leq \pm 0.4$ K 95 °C $\leq \pm 0.5$ K
Control accuracy:	± 0.2 K
Temperature-control speeds:	Heating rate: approx. 3 K/s, measured on the block Cooling rate: approx. 2 K/s, measured on the block
Number of programs:	max. 99 max. 10 on a personal card
Maximum number of cycles:	99
Dimensions:	Width: 26 cm Depth: 41 cm Height: 27 cm
Weight:	approx. 12.4 kg
Voltage/frequency:	230 V, 50–60 Hz / 115 V, 50–60 Hz / 100 V, 50–60 Hz
Power requirement:	500 W 500 W 500 W
Operating current:	2.6 A 5 A 6 A
Fuses:	2 x T 5 A, 250 V 2 x T 6.3 A, 250 V 2 x MT 8 A, 250 V
Overvoltage category:	П
Pollution degree:	2
Contamination level:	Ι
Operating conditions:	15 to 35 °C, 70 % rel. humidity
Storage conditions:	–20 to 70 °C, 85 % rel. humidity

The device is  $\bigcirc$  -approved, fulfills UL 3101-1 and CSA C 22.2 No. 1010.1.

Technical specifications subject to change!

Please use only the accessories recommended by Eppendorf. Using disposables which we have not recommended can reduce the precision, accuracy and life of the device. We do not honor any warranty or accept any responsibility for damage resulting from such action.

Order no.	Description
	Mastercycler <sup>®</sup>
5333 000.018 5333 000.026	230 V, 50–60 Hz 115 V, 50–60 Hz
5333 000.034	100 V, 50–60 Hz
5001 000 010	Mastercycler <sup>®</sup> gradient
5331 000.010 5331 000.045	230 V, 50–60 Hz 115 V, 50–60 Hz
5331 000.037	100 V, 50–60 Hz
	Accessories
0013 609.349	Fuse, time-lag, 5 A, 250 V (1 pc.) for 230 V
0013 565.333	Fuse, time-lag, 6.3 A, 250 V (1 pc.) for 115 V
0013 611.432	Fuse, semi time-lag, 8 A, 250 V (1 pc.) for 100 V
5331 220.002	<i>in situ</i> Adapter
5331 900.062	Operating manual
	for Mastercycler <sup>®</sup> and Mastercycler <sup>®</sup> gradient
5332 300.018	Personal card
5331 226.027	Personal card for temperature-validation system
5331 222.005	Temperature-validation system
5331 224.008	Temperature sensor for 0.2 ml tube positions
	Consumables
0030 124.332	PCR-tubes 0.2 ml, colorless (1000 pcs.)
0030 124.502	PCR-tubes 0.5 ml, colorless, thin-walled (500 pcs.)
0030 124.359	8-strip 0.2 ml PCR tubes, colorless, per 120 pcs. (= 960 tubes)
0030 121.023	Safe-Lock microcentrifuge tubes 0.5 ml, colorless (500 pcs.)
0030 128.648	Eppendorf twin.tec PCR Plate 96, skirted, colorless (25 pcs.)
0030 128.656	Eppendorf twin.tec PCR Plate 96, skirted, coloness (25 pcs.)
0030 128.680	Eppendorf twin.tec PCR Plate 96, skirted, red (25 pcs.)
0030 128.664	Eppendorf twin.tec PCR Plate 96, skirted, red (25 pcs.)
0030 128.672	Eppendorf twin.tec PCR Plate 96, skirted, blue (25 pcs.)
0030 128.575	Eppendorf twin.tec PCR Plate 96, semi-skirted, colorless (25 pcs.)
0030 128.583	Eppendorf twin.tec PCR Plate 96, semi-skirted, yellow (25 pcs.)
0030 128.613	Eppendorf twin.tec PCR Plate 96, semi-skirted, red (25 pcs.)
0030 128.591	Eppendorf twin.tec PCR Plate 96, semi-skirted, green (25 pcs.)
0030 128.605	Eppendorf twin.tec PCR Plate 96, semi-skirted, blue (25 pcs.)

	Consumables
0030 127.498	Cap Strips, (8-strips) (25 x 12 pcs.)
0030 127.650	Heat Sealing Film (100 pcs.)
0030 127.668	Peel-it-Lite, Heat Sealing Foil (100 pcs.)
0030 127.676	Pierce-it-Lite, Heat Sealing Foil (100 pcs.)
0030 127.480	PCR Film, adhesive (100 sheets)
0030 127.471	PCR Foil, adhesive (100 sheets)
0030 127.501	<i>in situ</i> Frames, 25 μl (100 pcs.)
0030 127.510	<i>in situ</i> Frames, 65 μl (100 pcs.)
0030 127.528	<i>in situ</i> Frames, 125 μl (100 pcs.)
0030 127.536	<i>in situ</i> Frames, 300 μl (100 pcs.)
	Recommended auxiliary equipment
5425 715.005	Adapter for 0.2 ml PCR tubes for 1.5/2.0 ml rotor (6 pcs.)*
5425 716.001	Adapter for 0.5/0.6 ml tubes for 1.5/2.0 ml rotor (6 pcs.)*
0030 127.153	Capping Aid, sealing PCR tubes and plates (1 pc.)
4830 000.017	Eppendorf Biomaster <sup>®</sup> 4830
	and Mastertips <sup>®</sup> for contamination-free pipetting
	according to the positive-displacement principle
	in the volume range from 1 $\mu l$ to 20 $\mu l$
	Biomaster Kit = 1 Biomaster and 96 Mastertips
0030 127.641	Foil Stripper
0030 124.243	Frame for work tray (set of 5)
5390 000.016	Heat Sealer 115 V / 60 Hz
5390 000.024	Heat Sealer 230 V / 50 Hz
3881 000.015	PCR-Cooler 0.2 ml Starter Set (1 x pink, 1 x blue)
3881 000.023	PCR-Cooler 0.2 ml pink
3881 000.031	PCR-Cooler 0.2 ml blue
0030 127.455	PCR Rack, random colors (10 pcs.)
0030 124.235	Work tray for 0.2 ml PCR tubes (set of 10)

### PCR Reagents

see Main Catalog

\* For centrifugation

Please use only the accessories recommended by Eppendorf. Using disposables which we have not recommended can reduce the precision, accuracy and life of the device. We do not honor any warranty or accept any responsibility for damage resulting from such action.

Order no.	Description
	Mastercycler®
5333 000.018	230 V, 50–60 Hz
5333 000.026 5333 000.034	115 V, 50–60 Hz 100 V, 50–60 Hz
5004 000 040	Mastercycler <sup>®</sup> gradient
5331 000.010 5331 000.045	230 V, 50–60 Hz 115 V, 50–60 Hz
5331 000.037	100 V, 50–60 Hz
	Accessories
0013 609.349	Fuse, time-lag, 5 A, 250 V (1 pc.) for 230 V
0013 565.333	Fuse, time-lag, 6.3 A, 250 V (1 pc.) for 115 V
0013 611.432	Fuse, semi time-lag, 8 A, 250 V (1 pc.) for 100 V
5331 220.002	<i>in situ</i> Adapter
5331 900.062	Operating manual
	for Mastercycler <sup>®</sup> and Mastercycler <sup>®</sup> gradient
5332 300.018	Personal card
5331 226.027	Personal card for temperature-validation system
5331 222.005	Temperature-validation system
5331 224.008	Temperature sensor for 0.2 ml tube positions
	Consumables
0030 124.367	PCR tubes, 0.2 ml, colorless (1000 pcs.)
0030 124.600	PCR tubes, 0.5 ml, colorless, thin-walled (500 pcs.)
0030 124.383	8-strip 0.2 ml PCR tubes, colorless, per 120 pcs. (= 960 tubes)
22363611	Safe-Lock microcentrifuge tubes, 0.5 ml, colorless (500 pcs.)
0030 128.648	Eppendorf twin.tec PCR Plate 96, skirted, colorless (25 pcs.)
0030 128.656	Eppendorf twin.tec PCR Plate 96, skirted, yellow (25 pcs.)
0030 128.680	Eppendorf twin.tec PCR Plate 96, skirted, red (25 pcs.)
0030 128.664	Eppendorf twin.tec PCR Plate 96, skirted, green (25 pcs.)
0030 128.672	Eppendorf twin.tec PCR Plate 96, skirted, blue (25 pcs.)
0030 128.575	Eppendorf twin.tec PCR Plate 96, semi-skirted, colorless (25 pcs.)
0030 128.583	Eppendorf twin.tec PCR Plate 96, semi-skirted, yellow (25 pcs.)
0030 128.613	Eppendorf twin.tec PCR Plate 96, semi-skirted, red (25 pcs.)
0030 128.591	Eppendorf twin.tec PCR Plate 96, semi-skirted, green (25 pcs.)
0030 128.605	Eppendorf twin.tec PCR Plate 96, semi-skirted, blue (25 pcs.)

# 14 a Ordering information for North America

	Consumables
0030 127.498	Cap Strips (8-strips) (25 x 12 pcs.)
0030 127.650	Heat Sealing Film (100 pcs.)
0030 127.668	Peel-it-Lite, Heat Sealing Foil (100 pcs.)
0030 127.676	Pierce-it-Lite, Heat Sealing Foil (100 pcs.)
0030 127.480	PCR Film, adhesive (100 sheets)
0030 127.471	PCR Foil, adhesive (100 sheets)
0030 127.501	<i>in situ</i> Frames, 25 μl (100 pcs.)
0030 127.510	<i>in situ</i> Frames, 65 μl (100 pcs.)
0030 127.528	<i>in situ</i> Frames, 125 μl (100 pcs.)
0030 127.536	<i>in situ</i> Frames, 300 μl (100 pcs.)
	Recommended auxiliary equipment
5425 715.005	Adapter for 0.2 ml PCR tubes for 1.5/2.0 ml rotor (6 pcs.)*
5425 716.001	Adapter for 0.5/0.6 ml tubes for 1.5/2.0 ml rotor (6 pcs.)*
0030 127.153	Capping Aid, sealing PCR tubes and plates (1 pc.)
22440501	Eppendorf Positive Displacement Pipette Model 4830
	1 Kit = 1 Pipette Plus 96 Mastertips
	100 Safe-Lock tubes (0.5 ml)
0030 127.641	Foil Stripper
0030 124.243	Frame for work tray (set of 5)
5390 000.016	Heat Sealer 115 V / 60 Hz
5390 000.024	Heat Sealer 230 V / 50 Hz
22510509	PCR-Cooler 0.2 ml Starter Set (1 x pink, 1 x blue)
22510541	PCR-Cooler 0.2 ml pink
22510525	PCR-Cooler 0.2 ml blue
0030 127.455	PCR Rack, random colors (10 pcs.)
0030 124.235	Work tray for 0.2 ml PCR tubes (set of 10)

### PCR Reagents

see Main Catalog

\* For centrifugation

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# EG-Konformitätserklärung EC Conformity Declaration

Das bezeichnete Produkt entspricht den einschlägigen grundlegenden Anforderungen der aufgeführten EG-Richtlinien und Normen. Bei einer nicht mit uns abgestimmten Änderung des Produktes oder einer nicht bestimmungsgemäßen Anwendung verliert diese Erklärung ihre Gültigkeit.

The product named below fulfills the relevant fundamental requirements of the EC directives and standards listed. In the case of unauthorized modifications to the product or an unintended use this declaration becomes invalid.

Produktbezeichnung, Product name:

Mastercycler<sup>®</sup> gradient 5331, Mastercycler<sup>®</sup> 5333

Produkttyp, Product type:

Thermocycler zur DNA-Vervielfältigung / Thermocycler for DNA amplification

Einschlägige EG-Richtlinien/Normen, Relevant EC directives/standards:

2006/95/EG, EN 61010-1, EN 61010-2-10/A1

2004/108/EG, EN 55011/B, EN 61000-6-1, EN 61000-3-2, EN 61000-3-3

Vorstand Board of Management: 21.05.2007

Projektmanagement, Project Management:



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