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THERMO HYBAID

PCR Express Thermal Cycler

USER INSTRUCTION MANUAL

Head Office

Thermo Hybaid
Action Court
Ashford Road
Ashford
Middlesex TW15 1XB

Telephone: +44 (0) 1784 425000
Free Phone UK: 0500 95 55 72
Facsimile: +44 (0) 1784 248085

STOCK CODE:
HB-PX-MAN

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PCR EXPRESS TEMPERATURE CYCLING SYSTEM
PCR License Registration

This instrument, Serial No. ..... is an Authorized Thermal Cycler. Its purchase price includes the up-front fee component of a license under the patents on the Polymerase Chain Reaction (PCR) process, which are owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd, to practise the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Perkin-Elmer or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications' licenses available from Perkin-Elmer. 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. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at The Perkin-Elmer Corporation, 850 Lincoln Centre Drive, Foster City, California 94404, USA. Tel No: + 415 638 5551. Fax No: +415 638 6071.

Perkin-Elmer does not guarantee the performance of this instrument.

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PCR EXPRESS TEMPERATURE CYCLING SYSTEM
Warranty

Thermo Hybaid guarantees that the PCR Express Temperature Cycling system you have received has been thoroughly tested and meets its published specification.

This guarantee is valid for 36 months only if the product and functions have been used according to the instruction manual. No liability is accepted for loss or damage arising from the incorrect use of the PCR Express Temperature Cycling system. Thermo Hybaid's liability is limited to the repair or replacement of the unit or refund of the purchase price at Thermo Hybaid's option. Thermo Hybaid is not liable for any consequential damages.

The tube thermistor assembly supplied with your PCR Express unit is guaranteed for 90 days.

Thermo Hybaid reserves the right to alter the specification of the PCR Express without prior notice. This will enable us to implement developments as soon as they arise.

The Thermo Hybaid PCR Express is for research use only.

Read the Instruction Manual carefully before using the PCR Express to ensure that you obtain the best possible results from the machine.

NB: The PCR Express should only be used by suitably qualified and trained people. If the PCR Express is not used as specified in this Manual, the protection provided by the equipment may be impaired.

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CHAPTER 1
PCR EXPRESS THERMAL CYCLER
Introduction

1.1 Overview

PCR Express is the essence of fast accurate, licensed thermal cycling. PCR Express features high capacity, high speed and sub-ambient blocks to perform oil-free thermal cycling with excellent dynamic uniformity and precision control of sample temperature.

1.2 PCR Express System



The PCR Express System comprises a Control Chassis and an Interchangeable Block Module. Each control chassis can operate one block module, which can be changed according to the needs of the sample format. There are six types of block module:

The 0.2ml Block Module & 0.2ml Gradient Block Module

This block can hold any of the following:

- 1 x OmniPlate 96 well plate
- 1 x OmniTube 96 single piece polypropylene plate

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- 96 x 0.2ml OmniStrip tubes
- 96 x 0.2ml individual OmniTubes

The 0.5ml Block Module & 0.5ml Gradient Block Module

This block can hold any of the following:

- 1 x 96 well plate (e.g. Costar Thermowell H)
- 96 x 0.3ml OmniStrip tubes
- 96 racked 0.3ml OmniTubes
- 48 standard 0.5ml individual OmniTubes.

The Flat Block Module

This block accommodates up to four standard microscope slides.

The 384 Well Block Module

This block accommodates 1 x OmniPlate 384 polypropylene plate.

Temperature Control Methods

The temperature cycling blocks for the system are of a specially coated precision-machined aluminium, ensuring the best possible fit of reaction tubes in the block. This ensures excellent contact between the tubes and the block, enabling rapid and accurate heat transfer from the block to the samples.

1. Active Tube Control

Accurate sample temperature control in 0.2ml and 0.5ml block types is achieved by Thermo Hybaid's **Active Tube Control** software. The tube thermistor probe monitors the temperature within a dummy sample tube and this information is fed back to precisely control the block temperature to achieve the optimum cycling profile.

2. Simulated Tube/Plate Control

Alternatively, **Simulated Tube Control** or **Simulated Plate Control** may be used for reactions when it is not appropriate to use the tube thermistor probe, for example in very small (<20µl) reactions or reactions in 96 Well or 384 Well OmniPlates or racked OmniTubes. The

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temperature control algorithm is similar to active tube control, but is based on calculated values for the sample temperature rather than values fed back by the tube thermistor.

3. Simulated Slide Control

For the *In Situ* module **Simulated Slide Control** should be selected. This operates on a similar principle to Simulated Tube Control, but in this case the algorithm is based on achieving the set temperatures at the top surface of a standard glass microscope slide (1mm thick).

Heating and Cooling

The PCR Express **sub-ambient** blocks are built to proven designs, providing an accurate and reliable thermal cycling system.

The sub-ambient aluminium block is heated and cooled by the latest in Peltier technology. With proven durability, the block modules excel in performing applications such as RAPD and Differential Display, which require cycling temperatures close to ambient. The block modules will control the temperature of the samples from 20°C to 99°C for cycling reactions in all reaction formats. In addition, static incubation steps may be performed as low as 4°C.

Programming and Operation

Cycling programs are simple to perform, using the combination of user-friendly screens and operating keys. During programmed operation, the display screens provide comprehensive information for the block including sample temperature, number of cycles completed, estimated time for completion, etc.

The PCR Express can thus be programmed to perform all types of temperature controlled reactions, from simple one step incubations, to complex multi-step temperature cycling protocols and temperature gradient experiments*. Three additional temperature control software options are available to the user in the Advanced Edit Menu: Temperature Ramping, Time Increment and Temperature Increment.

The unit has program space for up to 99 full cycling protocols including the pre-set protocols.

* With the gradient interchangeable block options.

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Pre-set Programs

The PCR Express is supplied with ten non-editable pre-set protocols stored as programs 90-99 in the F:HYBAID directory. These cover the most common thermal cycling techniques, and can be used to create customised protocols by editing (see Appendix VI).

Advanced Edit - Temperature Ramping

The temperature ramping (Ramp Rate) enables the rate of change of sample temperature (°C/second) to be artificially slowed down.

Under normal circumstances the temperature cycling times are very rapid, which minimises non-specific reactions. The rate of sample temperature change during temperature cycling is controlled so that it is as fast as possible without affecting the block uniformity and accuracy. In some instances, it may be advantageous to limit the rate of change of temperature, e.g. to allow limited extension of short or degenerate primers between primer annealing and DNA synthesis steps to stabilise the primer/template duplex.

Advanced Edit - Time/Temperature Increment

These features enable the time interval and/or the temperature of a specified programmed step to be increased or decreased with successive temperature cycles. It may be advantageous to increment the extension time interval to compensate for deterioration of enzyme activity in later cycles. Alternatively, temperature decrements can be used, e.g. in PCR Express cycling reactions where the annealing temperature is decreased with successive cycles.

Temperature Control

Every machine is calibrated using miniature thermistor probes located in tubes or attached to microscope slides. These are placed at several block positions simultaneously, ensuring the required temperatures and incubation times achieved are identical in all samples.

1.3 The Heated Lid

The unique auto-adjust Heated Lid enables the running of temperature cycling protocols without the need for paraffin or mineral oil overlays. Such vapour barriers are normally required to prevent evaporation before thermal cycling is complete. This system is designed to be compatible with most reaction sample formats, i.e. 0.5ml, 0.3ml, 0.2ml tubes and 96/384 well plates. In most cases, the experimental protocol is largely unchanged when switching to an oil free format.

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The Heated Lid operates by positioning a heated plate in contact with the top of the reaction tubes or wells. This heated plate then heats the air temperature at the top of each reaction mixture to a temperature that is permanently higher than the sample temperature. This elevated air temperature, relative to the sample temperature, minimises evaporation so that there is no condensation of the reaction mixture as it is repeatedly heated and cooled.

Opening the Lid

The lid is opened by lifting the light blue catch, and pushing it back.

The mechanism operates in 2 stages but through one continuous action; the first part of the action raises the heating plate within the machine, the second releases the catches at the front of the unit to raise the lid and expose the block recess.

Closing the Lid

To close, gently lower the dark blue lid and depress the light blue catch. At this point a 'click' should be heard as the catches are engaged. This action lowers the heated lid on to the samples and closes the unit, ensuring tight contact.

The height of the heating plate inside the Heated Lid is automatically adjusted as the lid is closed. The downward pressure can be adjusted using the set switch according to whether light pressure (300g for tubes) or greater force (3.5kg to maintain the seal on plates) is required.

The temperature of the heating plate is set at 115°C (max. surface temperature) with power control effected by the control chassis.

The heated plate inside the lid is switched on and off from within the program. The lid operates once a program is activated, with a heating time of typically 2 minutes before cycling commences. The unit can be operated with or without the lid being switched on (the lid is automatically deactivated in units fitted with flat block modules).

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1.4 Support Services

The PCR Express has been designed for reliability and for ease of maintenance. Thermo Hybaid continues to offer full service and technical support for all its products.

Telephone Contacts:

International Sales, Service and Technical Support	(UK) +44 (0)1784 425000
UK Sales and Technical Support	01784 425000/(Freephone) 0500 95 55 72
UK Service Support	01784 425000/(Freephone) 0500 49 22 43

Fax Contacts:

UK & International Sales, Service & Technical Support	(UK) +44 (0) 1784 248085

Alternatively, contact your local Thermo Hybaid subsidiary or authorised distributor.

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CHAPTER 2
PCR EXPRESS THERMAL CYCLER
Safety Precautions

General Safety Precautions

1. The PCR Express should only be used by suitably qualified and trained personnel. The unit should only be used for its intended purpose in accordance with the instructions and safety warnings contained within this manual.
2. Before use, ensure that the unit has been set to the appropriate mains voltage.
3. The PCR Express is a class 1 appliance. To minimize the risk of electric shock, the unit must be connected to a protective earth via the supplied mains cord.
4. Replacement fuses must be of the correct rated current, voltage and type.
5. Do not operate the unit in an explosive environment.
6. Do not operate the unit if it appears to be damaged or if a liquid or foreign object has entered the enclosure. Disconnect from the mains supply and contact an authorized service centre.
7. Do not attempt to dismantle the unit. To avoid the risk of personal injury and to ensure that the safety features of this unit are maintained, servicing should only be carried out by authorized service personnel.
8. The tops of the reaction vessels and the surfaces of the Heated Lid assembly (in particular the inner surfaces) can become very hot during normal operation. Touching the surfaces can cause burns. Do not touch the heated plate without safety gloves.
9. When positioning the unit ensure that there is no restriction to the power inlet. Special care should be taken not to obstruct the vent underneath the unit, for example loose Benchcote or sheets of paper.
10. When installing/removing the interchangeable block
    - Ensure that the power to the instrument is turned off.
    - The Block Heat Sink may be hot after use. Use gloves when interchanging blocks if the unit has been used recently.
11. This product is fitted with RFI suppression circuitry. Testing of the electrical insulation should only be carried out using a DC voltage. For more information please contact Thermo Hybaid.

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Symbols & Conventions

The PCR Express has been designed for safe operation. The following symbols appearing on the unit and their meanings should be noted.

I Indicates the ON position of the main power switch.

O Indicates the OFF position of the main power switch.



Consult the manual for further information.
Consulter les documents d'accompagnement.



WARNING: Indicates a heating hazard. Proceed with caution to avoid burn injury.

ATTENTION: *Surface chaude.*



SAFETY NOTE: This symbol indicates high voltage. Risk of electric shock.

AVERTISSEMENT: *Risqué de choc éléctrique.*

In addition, the following conventions are adopted in the manual in respect of indicating safety hazard:



SAFETY NOTE: indicates a potentially hazardous situation, which could result in death or serious injury.



WARNING: indicates a potentially hazardous situation, which could result in minor or moderate injury to the user or damage to the instrument.

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CHAPTER 3
PCR EXPRESS THERMAL CYCLER
Unpacking & Installation

3.1 Unpacking

Before unpacking the PCR Express, please make sure that the outer packaging is undamaged.

After unpacking, ensure all packaging and fixtures are retained, as the unit should always be transported in the original packing to avoid damage. Thermo Hybaid cannot accept responsibility for any damage incurred if the unit is incorrectly packed and transported.

3.2 Packing List

1. PCR Express Control Chassis
2. PCR Express Block Module
3. User Instruction Manual
4. Mains Lead
5. Control Thermistor Probe (0.2ml and 0.5ml gradient and standard block modules)
6. Control Thermistor Probe Extension Lead (0.2ml and 0.5ml gradient and standard block modules)
7. Specific Consumable Pack
8. RS232 Lead
9. Self Adhesive Unit Identity Label

NB: The *In Situ* Flat Block Module includes a humidity chamber.

If any item is missing or damaged, contact the UK Service Department, or your local Thermo Hybaid office/authorised distributor.

Ensure any padding between the plate inside the Heated Lid and the block is removed before using the instrument.

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3.3 Installation

Block Installation and Removal

1. Remove the PCR Express Control Chassis and Block Module from the packaging and place on the bench; do not connect to the mains supply immediately.

2. Open the lid (by lifting the light blue latch) to expose the block recess.

3. Supporting the block from the blue plastic moulding and the ring pull, position the back of the block on the two lugs on the chassis.

4. Lower the block, via the pull ring into the recess (see fig 3.1). Placing your thumb on the ring pull (just beneath the hole) firmly push downwards until you hear the block click into place.

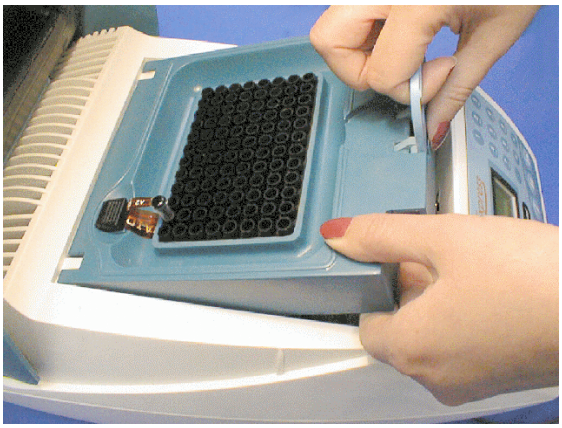
5. Do not force the block if this does not happen: remove the block and check for obstructions in the block recess. The following error screens will appear if the block has not been installed properly or you attempt to run a program without a block in place:

   SELF CHECK FAILED or SELF CHECK FAILED

   BLOCK NOT PRESENT BLOCK ID ERROR

Removal is the reverse procedure.

Figure 3.1 Installing the Block Module



Thermistor Positioning

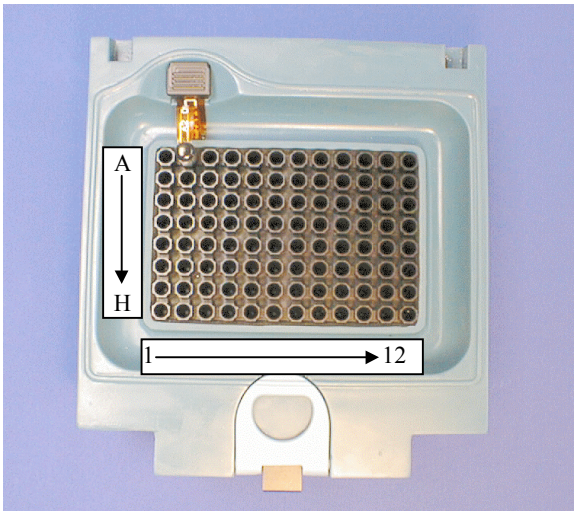
The control thermistor probe (when required) should be inserted into socket A2 to the left of the block towards the rear of the instrument (see fig 3.2).

Ensure that the tube contains the same volume of mineral oil as the sample reaction volume, and that the probe is immersed in the oil.

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Figure 3.2 Well Numbering Showing Tube Thermistor Positioning in A2



Choosing a Location

1. Where possible, avoid connecting the Control Chassis to a mains supply subject to mains voltage fluctuations, e.g. a socket shared by an ultracentrifuge or refrigerator.

2. In PCR Express systems, excess heat is removed from the system by a fan. Therefore, allow at least 8cm clearance between the sides of each module and any restriction to the flow of air. Allow 15cm of clearance at the back of the unit. The air intake is located underneath and air is expelled out of the back of the unit. Care should be taken not to obstruct the vent underneath the unit with, for example, loose Benchcote or sheets of paper. The unit must not be covered during operation.

3. All PCR Express instruments should be placed on a stable and level surface, out of direct sunlight and away from strong currents of hot or cold air. The heated lid should always be closed during temperature cycling, even if the lid is not switched on.

4. The PCR Express is intended for indoor use at an ambient temperature of 4-34°C in conditions of up to 80% humidity. These specifications have been calculated for operations at between 0 and 2000m altitudes.

Use in Cold Rooms

The PCR Express may be used in a cold room (down to 4°C). However, when removed to room temperature the instrument should be allowed to equilibrate for at least two hours before being switched on. If equilibration is not done, condensation may form within the unit and cause a short circuit.

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CHAPTER 4
PCR EXPRESS THERMAL CYCLER
Operating Instructions

4.1 Initial Start Up

(a) Switch on the PCR Express at the power switch at the rear of the unit. When first switched on the unit will complete a start up and self test routine.

(b) After 10 seconds a screen will display "PCR Express going through a self test procedure". This procedure need only be followed once a week. This can be by-passed by pressing the **CONTINUE** key when the initialisation screen is visible.

(c) The self-test routine checks the block thermistor, heating and cooling circuits and the heated lid. This takes approximately 2 minutes. (As the block will become hot during the self-test procedure, please keep the lid closed.)

4.2 Main Menu

After successful start up, the first menu to be displayed is the 'Main' Menu. It is possible to return to this menu at any time by pressing 'MENU'. The Main Menu displays the date, time and the status of the block (active, inactive, interrupted or active resumed).

All aspects of the programming are accessed through the Main Menu.

Table 4.1 Summary of Main Menu Functions

RUN	Select **RUN** to run an existing program.
MAN	Select **MAN** to run a manual static incubation.
PROG	Select **PROG** to edit, copy, view or erase a program.
OPTS	Select **SET UP** to alter the time, date, and power resume function and display brightness settings and to identify software version fitted. Select **CALC** to access the gradient calculator software (only relevant to gradient protocols).

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4.3 Operating Keys

The PCR Express software is designed to be intuitive, the following table gives a summary of the primary functions of each of the operating keys.

KEY	FUNCTION
ENTER KEY	- Selecting Menu and program choices.
MENU KEY	- Always moves to the Main Menu – (can be utilised as an "escape" key).
NUMBER KEYS	- Used for entering parameters and selecting programs.
ARROW KEYS	- Moving cursor between program positions. - Alphanumeric naming of programs/directories. - Used to toggle between options. - Used for rapid scrolling through programs and for moving between run screens.
STOP KEY	- Cancels instructions/operations/programs. - Used to delete programs & directories.
CONTINUE KEY	- Used to progress from "hold", "pause" or "self test" instructions. - Also used to access advanced edit functions.
PAUSE KEY	- Pauses a cycling program at current step.
BLOCK KEY	- Provides access to current/historical information on block status screens.

These functions are discussed fully in the following chapters.

4.4 Set Up Functions

Selecting SETUP at the Main Menu and pressing **ENTER** gives access to the SETUP menu. The SETUP menu also displays the current software version.

Clock

The time and date format of the clock display can be modified. To set the clock or alter the format, select CLOCK from the SETUP menu and press **ENTER**.

The American (MM/DD/YY) or European (DD/MM/YY) format can be selected using the ➔ key.

Press **ENTER** to move to the time/date setting fields. Use the number keys to enter time (hrs & mins) and day/month/year information. Press **ENTER** to return to the Main Menu.

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Power Failure Resume

If there is a power interruption during a run, the PCR Express will switch back on automatically. The unit can be set to resume a program (at the cycle and setpoint where the interruption occurred) or to abandon the program. Select POWER from the SETUP menu and press **ENTER**. The ABANDON or RESUME options can be selected using the ➔ key. Press **ENTER** to return to the Main Menu.

The instrument will record the duration of the interruption and the step, stage and cycle number at which it occurred.

(*Note that if there have been several breaks in the power supply, only the most recent interruption is recorded.)*

Display Adjustment

The display brightness can be adjusted under varying light conditions.

- Select DISPLAY from the SETUP menu, and press **ENTER**.
- Hold down ↑ or ↓ to increase or decrease the brightness observed on the screen.
- Press **ENTER** to save the current screen brightness and return to the Main Menu.

4.5 Running a Program

Running a pre-stored program is easy on the PCR Express.

- At the Main Menu, with the cursor on RUN, press **ENTER**.
- Select the source directory (e.g. F:HYBAID) and press **ENTER** then select program number to be run and follow the screen prompts.

Prior to starting the program, pressing the **MENU** key at any stage, will return to the Main Menu. More details can be found in Chapter 6.

4.6 Manual Operation

This option is used for static incubations when thermal cycling is not required, e.g. hot start protocols, probe denaturations, enzyme reactions. In this mode the unit will operate under block control (see Chapter 5 for details). The count-up timer will start when the block reaches temperature.

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1. Select MAN from the Main Menu and press **ENTER**.

2. Enter the name of the user or protocol if desired using the ↑ and ↓ arrows to scroll through the characters. Move to the next/previous character using the ← and → arrow keys.

3. Enter the temperature using the numeric keys. Press **ENTER** to continue.

4. Select the heated lid on (auto) or off using the → key.

5. Press **ENTER** to start the incubation. The screen displays the set temperature and actual temperature of the block together with the elapsed time.

NB: If the machine is started from 'cold', approximately two minutes will be required before the Heated Lid is at operating temperature (based on 20°C ambient and 230V supply. This may vary under different power conditions). Condensation may be experienced if samples are loaded before the lid is at operating temperature.

6. Press **STOP** twice to cancel the manual program.

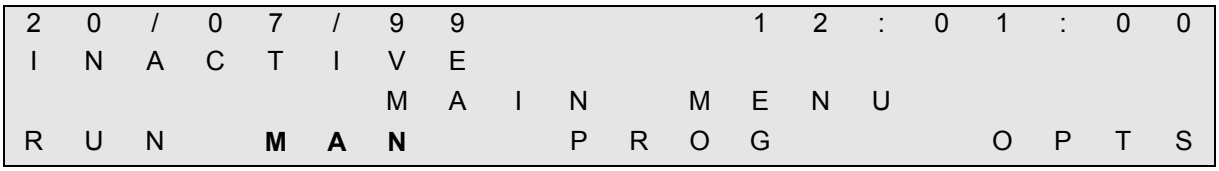
7. Press **CONTINUE** to change the set temperature.

(See Figure 4.1 on the following page)

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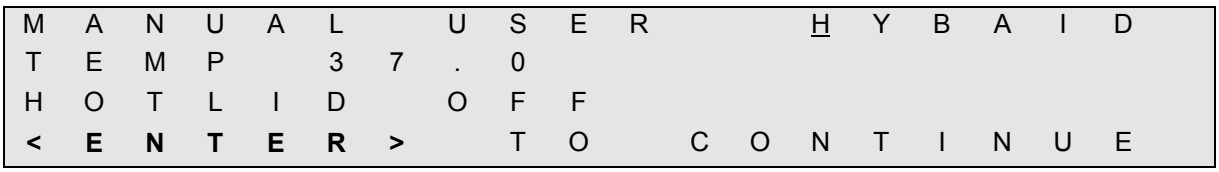
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Figure 4.1:Using the PCR Express for static temperature incubations (Manual control)



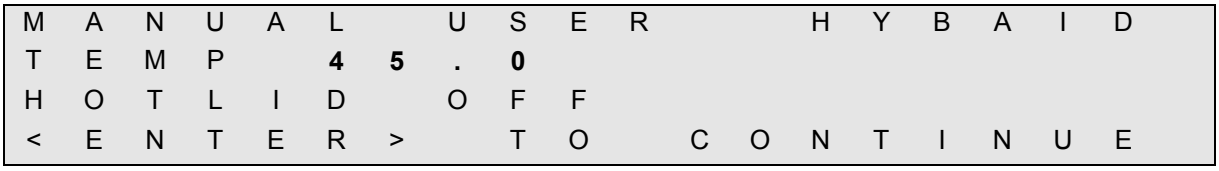
(1) Press **ENTER** to select the MANUAL mode.

↓



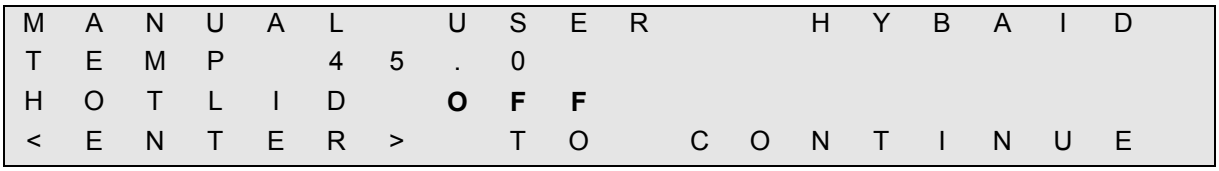
(2) Use ↑,↓,→, and ← to enter name then press **ENTER**.

↓



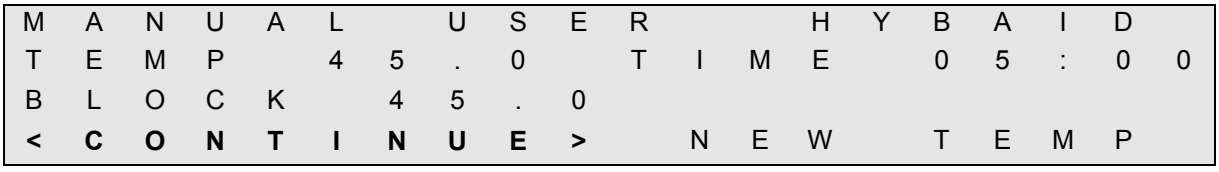
(3) Use number keys to enter the required temperature, then press **ENTER**.

↓



(4) Use → to toggle heated lid on (auto) or off, then press **ENTER**.

↓



(5) Press **CONTINUE** to change the setpoint temperature, or **STOP** to stop the incubation

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4.7 Programming Function

Programming PCR protocols of varying degrees of complexity can easily be created using PCR Express. As with all operations of the PCR Express, programming is accessed from the Main Menu. Press **MENU** to access the Main Menu screen.

Use the arrow keys to move the cursor to **PROG**, and then press **ENTER** to access the Program Menu. If at any time you wish to stop programming, press **MENU** to return to the Main Menu. Four options are available for programming:

1. **EDIT:** To alter the details of an **EXISTING** program or to create a completely **NEW** program.

   Note that if NEW is used, it will automatically move to the next available program number.

2. **COPY:** Use the screen prompts to copy between programs stored on the PCR Express.

3. **VIEW:** To check the details of a previously stored program.

4. **ERASE:** To erase the details of a program, setting the parameters to zero.

These are discussed in detail in Chapter 5.

4.8 Loading Samples and Heated Lid Operation

The PCR Express will produce identical cycling profiles whether 1 or 96 samples are to be analysed.

Loading Samples

The following guidelines maximise the uniformity and thermal transfer characteristics of the block:

- Load samples uniformly across the block rather than in clusters. Spreading the thermal load in this way maintains uniformity.
- If small numbers of samples are to be run, use dummy tubes to ensure that there is at least one tube in each quadrant of the block. This facilitates even heated lid pressure for good thermal transfer to the tubes.
- Care should be taken when matching block, consumable, control mode and sample number (as shown below).

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- Ensure tube caps are properly closed before loading the block.

Table 4.2 Consumable Compatibility Table

Type*	Control Method**	Block 0.5ml	Block 0.2ml	Notes
0.5ml	TUBE SIM TUBE	✓	X	Max. 48 tubes/block arranged in a diagonal pattern e.g. A2, B1, A4, B3, C2, D1 etc. (see also figure 3.2). Space out tubes for even loading.
0.3ml	TUBE SIM TUBE	✓	X	Max. 96 tubes/block. Space out strips for even loading.
0.2ml	TUBE SIM TUBE (including OmniTube 96, 96 well arrays)	X	✓	Max. 96 tubes/block Space out strips for even loading.
OmniPlate 96	SIM PLATE	?	✓	Seal using TD Tape/compression pad or OmniSeal mat for oil free operation (see also App II). Use on 0.5ml blocks may require optimisation**
U-Well 0.5ml Plates	SIM PLATE	✓	X	Sealing may be problematic for oil free operation TD Tape is the best option.

* Details of Thermo Hybaid's own consumable range are given in Chapter 9
** See Section 5.2

Heated Lid Operation

The Auto Adjust Heated Lid is simple to operate. In one action, the lid is closed and the heated plate lowered to its correct height and pressure setting.

No adjustment is necessary to compensate for different height consumables.

Set Switch

The Set Switch allows selection between a higher and a lower pressure setting. The switch is labelled "PLATE" and "TUBE".

- "PLATE" setting should be used when sealing 96/384 well plates with TD Tape/compression pad or OmniSeal TD Mats.
- "TUBE" setting should be used for all other consumables.

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CHAPTER 5
PCR EXPRESS THERMAL CYCLER
Programming the PCR Express

5.1 Introduction

The design of the PCR Express is such that programming it is easy. The memory will hold up to 99 complete thermal cycling protocols including 10 pre-programmed template programs (Nos. 90-99 detailed in Appendix VI). Programs are identified by a number (01-99) and a user defined name (up to 7 characters). Each program protocol type may then assign to a directory, enabling you to group programs by user name.

To enter a new program you simply

i) Create a new directory

ii) Name a program

iii) Enter protocol parameters

iv) Save the program

5.2 Directories

There are five directories A-E which can be individually named. The sixth directory, F is reserved for pre-set programs.

Directories can also be erased and renamed as required.

Once assigned to a directory, a program can be copied to another program space in either the same or a different directory.

Creating a New Directory

1. From the Main Menu select **PROG** and **ENTER**

2. Select **EDIT** and **ENTER**

3. Select **NEW** and **ENTER**

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4. Select **NEWDIR** and **ENTER**

5. Select the desired target directory (A-E) using the cursor keys and then **ENTER**.

6. You are now able to name the directory, using the ↑ and ↓ arrows to scroll through the characters or the alphanumeric keypad. Move to the next/previous character using the → and ← arrow keys. Press **ENTER** only when the name is complete. If no characters are entered, the default NO NAME will be entered.

5.3 Steps & Stages of a Program

The programs in the PCR Express are divided into 'stages' and 'steps' when displayed on the screen. In a simple PCR program, a stage typically includes three steps where each step refers to the temperatures and times within the protocol. E.g. Step 1: 95°C for 30 seconds, Step 2: 55°C for 30 seconds, Step 3: 72°C for 30 seconds. In addition each stage can be repeated for up to 99 cycles, and a 'hold' added to the end of the stage.

The PCR Express allows you to program up to **10 separate stages each with up to 10 separate steps.** This means that even the most complex thermal cycling protocol may be saved in a single program space.

5.4 Creating a New Program

Using the NEW Program Option

Figure 5.1

2	0	/	0	7	/	9	9				1	4	:	1	5	:	0	7
I	N	A	C	T	I	V	E											
				M	A	I	N				M	E	N	U				
R	U	N			M	A	N			**P**	**R**	**O**	**G**			O	P	T	S

(1) Move cursor to **PROG**, then press **ENTER** to select.

↓

2	0	/	0	7	/	9	9					1	4	:	1	5	:	0	7
						I	N	A	C	T	I	V	E						
				P	R	O	G	R	A	M		M	E	N	U				
E	**D**	**I**	**T**		C	O	P	Y		V	I	E	W		E	R	A	S	E

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(2) Move cursor to EDIT, COPY, VIEW or ERASE as required (in this case **EDIT**), then press **ENTER** to select.

↓

```
20/07/99                14:15:09
                    INACTIVE
PROGRAM EDITING MENU
EXISTING          NEW
```

(3) Move the cursor **NEW** program then press **ENTER** to select.

↓

```
20/07/99                14:15:09
                    INACTIVE
                    NEW MENU
NEWPROG             NEW DIR
```

(4) Move the cursor to **NEWPROG** and press **ENTER** to select.

↓

```
SELECT DIRECTORY:
  A:ANITA         D:THOMAS
  B:ALISON        E:DIANA
  C:DAVE          F:HYBAID
```

(5) Move the cursor to desired target directory and press **ENTER** to select.

↓

```
NEW              A:05      PCR     60C
STAGE    01              STEP    01
TEMP    00:0
TIME    0:00:00
```

(6) The instrument allocates a program number of the next available program space. You are now able to enter the name of the user or protocol, using the ↑ and ↓ arrows to scroll through the characters or the alphanumeric keypad. Move to the next/previous character using the → and ← arrow keys. Press **ENTER** only when the name is complete. If no characters are entered, the default NO NAME will be entered.

Note: *If there are no free program spaces the PCR Express will prompt you to overwrite an existing program.*

↓

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N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		0	1				S	T	E	P		0	1		
T	E	M	P		**9**	**5**	:	**0**											
T	I	M	E		**0**	**:**	**0**	**5**	:	**0**	**0**								

(7) Enter temperature (e.g. 95°C) and time (hr:min:sec), as required. Use ➔ to move across menu, or ➓ to advance to the next step. The **ENTER** key can also be used to move to the next step of the protocol.

Note: The arrow keys can be used to scroll backward or forward to correct a mistake. The program will not allow you to advance to step 2 until a valid temperature and time have been entered.

Continue to enter the remaining times and temperatures of this stage. If none are required at the stage of the protocol, press **ENTER** (leaving each parameter at 0:0) until you access the number of cycle's screen.

N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		N	U	M	B	E	R						0	1	
N	U	M	B	E	R		O	F		C	Y	C	L	E	S		**0**	**1**	
H	O	L	D		T	E	M	P			0	0	.	0					

(8) Enter the number cycles for this stage using the alphanumeric keys. A hold step can also be programmed after each stage. This is particularly useful for 'hot start' protocols. If a hold step is not required, press **ENTER** to reach the next stage.

↓

N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		0	2				S	T	E	P		0	2		
T	E	M	P		**9**	**5**	:	**0**											
T	I	M	E		**0**	**:**	**0**	**0**	:	**3**	**0**								

(9) Continue adding steps as required for stage 1 (max. 10 steps per stage).

↓

N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		0	1				S	T	E	P		0	2		
T	E	M	P		**6**	**0**	:	**0**											
T	I	M	E		**0**	**:**	**0**	**0**	**:**	**3**	**0**								

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↓

N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		0	1				S	T	E	P		0	2		
T	E	M	P		**7**	**2**	:	**0**											
T	I	M	E		0	:	0	0	:	**3**	**0**								

(10) Press **ENTER** (or ↓ key) with each parameter at zero to finish the last stage of your protocol.

↓

N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		N	U	M	B	E	R						0	1	
N	U	M	B	E	R		O	F		C	Y	C	L	E	S		**3**	**0**	
H	O	L	D		T	E	M	P			0	0	.	0					

(11) Enter the number of cycles. Enter a hold temperature if required. Press **ENTER** to go to the next stage.

↓

N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		0	3				S	T	E	P		0	1		
T	E	M	P		7	2	:	0											
T	I	M	E		**0**	**:**	**0**	**5**	**:**	**0**	**0**								

(12) Enter any additional stages and steps you require up to a maximum of 10. Press **ENTER**.

↓

N	E	W						A	:	0	5		P	C	R		6	0	C
S	T	A	G	E		N	U	M	B	E	R						0	2	
N	U	M	B	E	R		O	F		C	Y	C	L	E	S		**0**	**1**	
H	O	L	D		T	E	M	P			0	0	.	0					

(13) Enter the number of cycles. Enter a hold temperature if required. If a hold temperature is not entered the instrument will cool to room temperature once the protocol is finished. Press **ENTER** to access the SAVE screen.

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↓

S	A	V	E				A	:	0	5		P	C	R		6	0	C
<	**E**	**N**	**T**	**E**	**R**	>	T	O		S	A	V	E					
<	M	E	N	U	>		T	O		A	B	A	N	D	O	N		

(14) **Press ENTER to save the program.**

5.5 Editing an Existing Program

When editing a program and saving as a different program, the COPY function should first be used to save the program to a different program space (see Section 5.7) and then the copy should be edited.

The program name can be edited or deleted at this stage although it will be saved unchanged if the enter key is pressed without changing the data.

Advanced editing features (adjusting ramp speeds, changing time/temperature with successive cycles) can be accessed by pressing the **CONTINUE** key at the relevant step. (See section 5.9.)

It is possible to edit programs whilst the PCR Express is running a different protocol.

Figure 5.2 Editing an existing program

2	0	/	0	7	/	9	9					1	4	:	1	5	:	0	7
I	N	A	C	T	I	V	E												
				M	A	I	N					M	E	N	U				
R	U	N			M	A	N			**P**	**R**	**O**	**G**			O	P	T	S

(1) To edit and overwrite existing programs select **PROG** from the Main Menu.

↓

2	0	/	0	7	/	9	9					1	4	:	1	5	:	0	7
						I	N	A	C	T	I	V	E						
				P	R	O	G	R	A	M		M	E	N	U				
E	**D**	**I**	**T**		C	O	P	Y		V	I	E	W		E	R	A	S	E

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(2) Select **EDIT** from the Program Menu.

↓

```
20/07/99                14:15:09
                      INACTIVE
PROGRAM EDITING MENU
EXISTING NEW
```

(3) Select **EXISTING** from the Program Editing Menu.

↓

```
SELECT DIRECTORY:
  A:ANITA         D:THOMAS
  B:ALISON        E:DIANA
  C:DAVE          F:HYBAID
```

(4) Move the cursor to the source directory and press **ENTER** to select.

↓

```
EDIT                A:05    NO    NAME


<ENTER> TO CONTINUE
```

(5) The keypad numbers or ↑↓ keys can be used to select the number of the program to be edited.

↓

```
EDIT                A:05    PCR   60C
STAGE   01                  STEP  01
TEMP    95:0
TIME    0:05:00
```

(6) Use the cursor keys to edit the program name. Press **ENTER** to proceed.

↓

```
EDITING             A:05    PCR   55C
STAGE   01                  STEP  01
TEMP    94:0
TIME    0:02:00
```

(7) Use → to move across the menu, and ↓ **or ENTER** to advance through the fields. Time and temperature parameters can be altered using the numerical keys as required.

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Note: To increase the number of steps in a stage, simply input values into the zero time/temperature fields. To decrease the number of steps in a stage just input the new data over existing time/temperature values.

↓

```
EDITING A:05 PCR 55C
STAGE 01     STEP 02
TEMP 00:0
TIME 0:00:00
```

(8) To complete editing stage 1, press **ENTER** with each parameter set at zero.

↓

```
EDITING A:05 PCR 55C
STAGE NUMBER 01
NUMBER OF CYCLES 01
HOLD TEMP 00.0
```

(9) Enter any changed cycle number or hold temperature values. Press **ENTER** to proceed to the next stage.

↓

```
NEW A:05 PCR 55C
STAGE 02     STEP 01
TEMP 94:0
TIME 0:00:20
```

(10) Edit steps in further stages as required. Press **ENTER** with each parameter at zero to access the save screen.

↓

```
SAVE A:05 PCR 55C
<ENTER> TO SAVE
<MENU> TO RETAIN
     PROGRAM
```

(11) Press **ENTER** to save changes.

5.6 Viewing an Existing Program

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It is possible to view existing programs without altering the information contained within them.

1. Select **PROG** from the Main Menu, **VIEW** from the program menu and (using the ← and → keys) enter the source directory.

2. Use the **ENTER** key and ↑ and ↓ arrows to move through the program, and **CONTINUE** to enter and leave the Advanced Edit information.

3. The **MENU** key closes the view screen, and returns to the Main Menu.

5.7 Copying Programs

Copying programs is necessary in, for example, modifying the pre-set programs described in Appendix VI. To do this select **COPY** from the Program Menu and press **ENTER**.

Enter the directory (using ← and → to select) and number of the source program (or use ↑ and ↓ to locate the source program) and press **ENTER**. Enter the directory and number of the target program (or scroll using ↑ and ↓) to locate a target program space. Press **ENTER** to copy the program. Note that any existing program data in the target program space will be overwritten.

5.8 Erasing a Program

Programs can be erased to vacate program space for future use.

From the Program Menu, select **ERASE** and press **ENTER**.

Enter the directory (using ← and → to select) and number of the program to be erased (or scroll using ↑ and ↓) and press **ENTER** to erase the program. Confirm erasure of the program by pressing **STOP** as prompted or **MENU** to keep the program.

5.9 Advanced Edit Features

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The Advanced Edit features allow the creation of more complex cycling protocols in order to enhance experimental data. It is possible to program:

- An incrementation or decrementation of both time and temperature on a cycle-by-cycle basis.

- The rate at which the temperature changes between two given temperatures.

Advanced Edit features are accessed by pressing the **CONTINUE** key at the temperature step at which the function is to take effect. If Advanced Edit data already exists for any step - **<ADV>** is indicated on the screen.

Enter and change Advanced Edit parameters using the number, arrow and **ENTER** keys. The Advanced Edit feature can also be accessed from the normal EDIT program menu options if Advanced Edit parameters are to be added to an Existing program.

If desired, the Advanced Edit feature can be deleted for the current step. To do this, set all the parameters back to zero. The **<ADV>** message will then not be shown in the temperature/time set up screen, denoting that no Advanced Edit functions are operating.

Time Advanced Edit Worked Example

The user can specify the increment/decrement per cycle for each program stage.

Time increments can be used for example in **high cycle number reactions** to allow longer for enzyme action with successive cycles.

Below is an example of a protocol where the extension step is fixed for the first 15 cycles of a 25-cycle program and increased by 5 seconds/cycle for the next 10 cycles, to compensate for loss of enzyme activity:

Stage 1: *Enter parameters to create:*

95°C - 30s

55°C - 30s *x 14 Cycles*

72°C - 30s

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Stage 2: *Enter parameters to create:*

95°C - 30s

55°C - 30s *x 11 Cycles*

72°C - 30s + 5s/cycle

Figure 5.3 Programming Time Increments

E	D	I	T	I	N	G		A	:	0	5		P	C	R		5	5	C
S	T	A	G	E		0	2				S	T	E	P		0	3		
T	E	M	P		7	2	:	0											
T	I	M	E		0	:	0	0	:	2	0								

(1) In the EDIT mode (NEW or EXISTING) move to the temperature step of interest.

↓

G	R	A	D		0	0						S	T	A	G	E		0	2
R	A	M	P			0	.	0	0			S	T	E	P			0	3
T	E	M	P		I	N	C		0	:	0	0							
T	I	M	E		I	N	C		0	:	0	0							

(2) Press **CONTINUE** to access the Advanced Edit screen.

↓

G	R	A	D		0	0						S	T	A	G	E		0	2
R	A	M	P			0	.	0	0			S	T	E	P			0	3
T	E	M	P		I	N	C		0	:	0	0							
T	I	M	E		I	N	C		0	:	0	**5**							

(3) Using the cursor, move through the fields until you reach the desired parameter. Use the → to toggle between Increase and Decrease in set point time/cycle. Enter the amount by which the set point time per cycle should change (min:sec) using the number keys. (E.g. an increase of 5 sec per cycle).

Note: It is possible to alter both the RAMP speed and TEMP increment/decrement parameters in this screen using the same keys. These altered parameters would also be executed, simultaneously during your experiment.

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↓

E	D	I	T	I	N	G		A	:	0	5		P	C	R		5	5	C
S	T	A	G	E		0	2				S	T	E	P		0	3		
T	E	M	P		7	2	:	0											
T	I	M	E		0	:	0	0	:	2	0				<	A	D	V	>

(4) Using ↓ or **ENTER** to get back to the Editing screen. **<ADV>** now appears on the screen indicating an Advanced Edit function has been programmed.

Temperature Advanced Edit Worked Example

Temperature decrements can be used (for example in **touchdown cycling reactions)** where the annealing temperature is decreased with successive cycles.

This example shows a protocol where the annealing step is fixed for the first 5 cycles of a 25 cycle program and decreases by 1.0°C/cycle for the next 15 cycles, to reduce specificity and increase yield as product accumulates (a "touchdown" protocol).

Stage 1: *Enter parameters to create:*

95°C - 30s

65°C - 30s *x 4 Cycles*

72°C - 30s

Stage 2: *Enter parameters to create:*

95°C - 30s

65°C - 30s – 1.0°C/cycle *x 16 Cycles*

72°C - 30s

Stage 3: *Enter parameters to create:*

95°C - 30s

50°C - 30s *x 5 Cycles*

72°C - 30s

Figure 5.4 Programming Temperature Decrements

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E	D	I	T	I	N	G		A	:	0	5		P	C	R		T	D
S	T	A	G	E		0	2				S	T	E	P		0	3	
T	E	M	P		6	5	:	0										
T	I	M	E		0	:	0	0	:	3	0							

(1) In the EDIT mode (NEW or EXISTING) move to the temperature step of interest.

↓

G	R	A	D		0	0						S	T	A	G	E		0	2
R	A	M	P			0	.	0	0			S	T	E	P			0	3
T	E	M	P		D	E	C		**1**	.	**0**	**0**							
T	I	M	E		I	N	C		0	:	0	0							

(2) Press **CONTINUE** to access the Advanced Edit screen. Move through the screen until you reach the TEMP field using the ↓ key. Use → to toggle between Increase and Decrease in set point temp/cycle. Enter the desired value by which the set point temp per cycle should change (°C) using the number keys (E.g. 1.0°C decrease per cycle).

↓

E	D	I	T	I	N	G		A	:	0	5		P	C	R		T	D
S	T	A	G	E		0	2				S	T	E	P		0	3	
T	E	M	P		6	5	:	0										
T	I	M	E		0	:	0	0	:	3	0				**<**	**A**	**D**	**V**	**>**

(3) Move back to the main step screen using the ↓ key. **<ADV>** will now appear on screen.

Changing Ramp Rates

Ramp rates can be altered in the Advanced Edit screen (refer to Figs.5.4 and 5.5). The default ramp rate (0.00°C/sec) is "as fast as possible". The settable range is 0.01°C/sec to 9.99°C/sec, with the maximum practical setting being 3.00°C/sec with current technologies. The data entered refers to the ramp rate to the CURRENT step from the PREVIOUS temperature set point.

For example, cycle sequencing of some targets requires the use of degenerate primers and a ramp rate set at 1°C/second between the annealing temperature and extension temperature.

5.10 Gradient Feature

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This feature is only available to those who have purchased a 0.2ml or 0.5ml gradient block for temperature gradient cycling. If an attempt to run a gradient program on a non-gradient block the following screen will appear:

T	H	I	S		P	R	O	G	R	A	M									
R	E	Q	U	I	R	E	S		A		G	R	A	D	I	E	N	T		
B	L	O	C	K		T	O		B	E		F	I	T	T	E	D			
<	M	E	N	U	>		T	O		R	E	S	T	A	R	T				

Customers who have purchased a PCR Express prior to 1 December 1998, ensure that:

- **Software version 2.0 or later (for use with the PCR Express gradient block) has been loaded on to your PCR Express chassis.**
- **Your PCR Express chassis has received a small wiring modification from a Thermo Hybaid Service Engineer.**

If these modifications have not been performed on your PCR Express chassis then your gradient block will not function. If you are unsure whether these adjustments have been made to your PCR Express chassis, please contact your local Thermo Hybaid office.

For customers who have purchased a PCR Express after 1 December 1998, all PCR Express chassis have been modified and are gradient compatible.

The Gradient feature is also accessed through the Advanced Edit function. Simply press the CONTINUE key from the annealing step in the main programming menu to access the Advanced Edit screen. After a gradient spread value has been entered in the GRAD section the number will be displayed on the main programming screen. E.g. **<G:10>**

The following screen will be displayed when attempting to run a program created under a previous version of the software, which is incompatible with the new program format in v 2.0 software. The following screen will only appear if an existing program contains time increments/decrements. This existing program should be edited by returning to the MAIN MENU and using **PROG-EDIT-EXISTING** to check that all steps (including other advanced edit functions) are re-entered as required.

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P	R	O	G	R	A	M		F	O	R	M	A	T		E	R	R	O	R
P	L	E	A	S	E		C	O	R	R	E	C	T						
B	E	F	O	R	E		R	U	N	N	I	N	G						
<	M	E	N	U	>		T	O		A	B	A	N	D	O	N			

For information on the Gradient Calculator function, refer to section 5.13.

Gradient Programming Worked Example

E	D	I	T	I	N	G		A	:	0	5		G	R	A	D			
S	T	A	G	E		0	2				S	T	E	P		0	2		
T	E	M	P		4	5	:	0											
T	I	M	E		0	:	0	0	:	2	0								

(1) In the EDIT mode (NEW or EXISTING) move to the annealing temperature step. The annealing temperature entered in this step will be the **lowest** temperature within the gradient. You must enter a time value as well in order to access the Gradient function in the Advanced Edit screen. Press CONTINUE to access the Advanced Edit screen.

↓

G	R	A	D		**1**	**5**						S	T	A	G	E		0	2
R	A	M	P			0	.	0	0			S	T	E	P			0	3
T	E	M	P		I	N	C		0	:	0	0							
T	I	M	E		I	N	C		0	:	0	0							

(2) Enter your required gradient range from 1-15°C.

↓

E	D	I	T	I	N	G		A	:	0	5		G	R	A	D			
S	T	A	G	E		0	2				S	T	E	P		0	2		
T	E	M	P		4	5	:	0						**<**	**G**	**:**	**1**	**5**	**>**
T	I	M	E		0	:	0	0	:	3	0								

(3) The program editing screen will now display the gradient spread depicted as **<G:15>**. If a time increment/decrement has been activated then <ADV> will also be displayed on this screen. Proceed though the programming as discussed in Figure 5.2.

Invalid Gradient Parameters

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If the combination of the Set Annealing Temperature and Gradient results in the block operating outside the temperature limits of 30 to 70°C a 'TEMP/GRAD INVALID' message will briefly be displayed in place of the entered value. E.g. SET TEMP = 70°C and GRAD = 10°C. The SET TEMP will need to be re-entered.

If a temperature is entered that exceeds the 15°C gradient range then a '??' error message will appear beside the GRAD parameter. A gradient range from 1 – 15°C will need to be re-entered.

Gradient Calculator Function

Once you have run your samples on an agarose gel, the annealing temperature that corresponds to your optimum gel result can be determined using the Gradient Calculator function. Refer to the following figure for an illustration on how to utilise this function.

Figure 5.5 Gradient Calculator Worked Example

```
20/07/99                    14:15:07
INACTIVE
          MAIN MENU
RUN       MAIN      PROG    OPTS
```

1. To access the Gradient Calculator move the cursor to OPTS in the MAIN MENU and press ENTER.

↓

```
20/07/99                    14:15:07
INACTIVE
          OPTIONS MENU
       SETUP      CALC
```

2. Move the cursor to CALC and press ENTER.

↓

```
GRADIENT CALCULATOR
BLOCK TYPE    0.2ml
```


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SET TEMP	**45.0**
GRADIENT	**15**

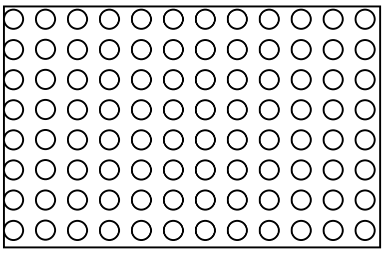
3. Specify the block type you have used to optimise your annealing temperatures by using the ← and → keys. Enter the annealing temperature and gradient range used in your optimisation experiment.

GRADIENT CALCULATION	
C1 : 45.4	C4 : 47.7
C2 : 45.8	C5 : 49.4
C3 : 46.4	C6 : 51.4

GRADIENT CALCULATION	
C7 : 53.3	C10 : 59.0
C8 : 55.3	C11 : 59.7
C9 : 57.6	C12 : 60.2

4. The well temperatures are displayed in sequence, on two screens. Each column temperature is represented by C1, C2…

E.g.



C1 C2 C3 C4……

5. Press **ENTER** to access the second screen or use the ↑ and ↓ keys to move back and forth between the screens. Press **ENTER** to return to the main menu.

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CHAPTER 6

PCR EXPRESS THERMAL CYCLER

Running a Program on the PCR Express

6.1 The RUN Option

The RUN option in the Main Menu enables you to:

- Run a previously stored program.
- Choose the method of temperature control used whilst the program is running.
- Choose whether to run your samples oil free i.e. Heated Lid option.
- Once a program is selected, the program name will be displayed and lid preheating (if selected) will commence.

If a program is selected and an inappropriate temperature control method is used (e.g. tube control or simulated tube control for an In Situ interchangeable block module; simulated slide for an 02/05 module) then a warning message will appear and the program will be run under the correct mode of control.

6.2 Running a Program on the PCR Express – Short Instructions

Figure 6.1 shows how to run a previously stored protocol. For further information on temperature control and heated lid functions refer to the sections towards the end of this chapter. It is possible to edit programs whilst the PCR Express is running a different protocol (see Section 5.5).

Figure 6.1: Running a Program on the PCR Express

2	0	/	0	7	/	9	9					1	4	:	1	5	:	0	7
						I	N	A	C	T	I	V	E						
				M	A	I	N					M	E	N	U				
R	**U**	**N**			M	A	N			P	R	O	G			O	P	T	S

1. Press ENTER to select the RUN mode from the MAIN MENU.

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↓

S	E	L	E	C	T		D	I	R	E	C	T	O	R	Y	:	
	A	:	A	N	I	T	A			D	:	T	H	O	M	A	S
	B	:	A	L	I	S	O	N		E	:	D	I	A	N	A	
	C	:	D	A	V	E				F	:	H	Y	B	A	I	D

2. Use the ↑ and ↓ keys to select the source directory.

↓

R	U	N					A	:	0	1		N	O		N	A	M	E
H	O	T	L	I	D								A	U	T	O		
L	O	A	D	I	N	G		A	L	A	R	M		O	F	F		
E	N	D		R	U	N		A	L	A	R	M		O	F	F		

3. Enter the program number using the ↑ and ↓ keys, and then press **ENTER.**

↓

R	U	N					A	:	0	2		H	Y	B	A	I	D
H	O	T	L	I	D								O	N			
L	O	A	D	I	N	G		A	L	A	R	M		O	F	F	
E	N	D		R	U	N		A	L	A	R	M		O	F	F	

4. Select the heated lid operating method, loading and end run settings, using the → and pressing **ENTER.**

↓

R	U	N					A	:	0	2		H	Y	B	A	I	D
							T	U	B	E							
P	R	E	S	S		▸		T	O		C	H	A	N	G	E	
<	E	N	T	E	R	>		T	O		C	O	N	T	I	N	U	E

5. Select the control mode using the → and pressing **ENTER.**

↓

P	R	O	G	R	A	M		A	:	0	2		H	Y	B	A	I	D
L	I	D		P	R	E	H	E	A	T		B	E	F	O	R	E	
A	U	T	O		S	T	A	R	T									
<	S	T	O	P	>		T	O		A	B	O	R	T				

6. Cycling commences if the heated lid is selected OFF. If it is ON then the lid will preheat.
   If MANUAL start is selected press ENTER to commence cycling.
   If AUTO start is selected then it will start automatically.

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6.3 Example Run Screens

When a program is running three different run screens can be displayed. These contain information about the progress of the run together with the temperatures achieved during the run. This allows the user to monitor the performance of the PCR Express and to determine the time of the end of the program. These can be accessed by pressing the ↑ and ↓ arrows, to scroll through each screen in turn.

These screens can be accessed from the Main Menu by pressing the BLOCK key.

Screen 1

R	U	N	N	I	N	G		A	:	0	2		H	Y	B	A	I	D	
T	E	M	P			9	5	.	0										
B	L	K				9	6	.	2							T	I	M	E
T	U	B	E			9	5	.	2						0	0	:	0	7

The current block performance screen displays the following:

- Program number and name.
- Current block and tube temperatures.
- Set point (programmed) temperature.
- Time remaining at current setpoint.

Screen 2

R	U	N	N	I	N	G		A	:	0	2		H	Y	B	A	I	D	
S	T	A	G	E		0	1			S	T	E	P		0	2			
C	Y	C	L	E		1	1	.	O	F		3	0						
M	A	X			9	7	.	4			M	I	N			4	4	.	6

The program progress and performance screen displays the following:

- The current stage and step of the running program.
- The total number of cycles completed and remaining.
- The maximum and minimum temperatures achieved during the run in the respective control mode.

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Screen 3

R	U	N	N	I	N	G		A	:	0	2		H	Y	B	A	I	D
C	O	N	T	R	O	L			T	U	B	E						
T	I	M	E		N	O	W		1	0	:	4	3					
P	R	O	G	R	A	M		E	N	D		1	2	:	0	3		

The estimated run time screen displays the following:

- Temperature control method.
- Time now.
- The calculated run end time (an estimated value which is updated throughout the run).

Screen 4 (During a gradient step)

R	U	N	N	I	N	G		A	:	0	2		H	Y	B	A	I	D
T	E	M	P			4	5	.	0	/	5	5	.	0				
B	L	K				4	5	.	2	/	5	5	.	1		T	I	M	E
T	U	B	E			4	5	.	1						0	0	:	0	7

The gradient run screen displays the following:

- TEMP 45.0 = Annealing temperature programmed.
- TEMP 55.0 = Highest annealing temperature programmed.
- BLK = Real time gradient range.
- TUBE 45.1 = Actual sample temperature in position A2 as monitored by the tube thermistor.

Power Failure Screen

This additional screen appears if the run has been interrupted by a power failure or power fluctuation severe enough to affect the unit. It contains the following information: -

- The duration of the power failure.
- The stage in the run when the power failure occurred.

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End of Run Screen

The PCR Express displays a further screen at the end of the run, this provides the following:

- The program name and number.
- The total run time.
- The maximum and minimum temperatures recorded during the run.

Block Idle Screen

If **BLOCK** is pressed and there is no run in progress, the block idle screen is displayed.

Error Screens

A number of error screens may be displayed if a fault has been detected in the unit. Contact the Thermo Hybaid Service Department or your local supplier for advice before attempting to use the instrument further.

6.4 Temperature Control Options

Crucial to the accurate operation of a thermal cycler is an understanding of the temperature control methodology.

Active Tube Control (TUBE)

Recommended for all reactions above 20µl volume in 0.5ml and 0.2ml tubes. This type of control uses the remote thermistor probe mounted in an appropriate tube. A volume of mineral oil equivalent to the total volume in the reaction tubes (including any oil overlay) must be present in the control tube **(do not use aqueous solutions with the control tube thermistor)**.

The thermistor acts as a simple mimic, monitoring the sample temperatures as it changes during cycling, feeding back this information to the PCR Express processor. This feedback allows the unit to respond to the sample temperature ensuring that the samples achieve the exact temperatures and times programmed. To bring the sample to temperature rapidly, the block is heated/cooled beyond the set temperature for the sample (*Figure 6.2*). When designing/transferring to a tube control program it is essential to understand the difference

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between tube control and block control on a conventional temperature cycling machine. With tube control, the actual samples are held at the programmed temperature for the programmed time. With block control, either on the PCR Express or a thermal cycler without tube control, there will be a lag between the block reaching target temperature and the sample reaching target temperature. Thus when transferring protocols from a block control machine the incubation times may be reduced by up to 50%, and in some cases the temperatures adjusted slightly.

For Tube Control reactions, check that the tube thermistor is connected and located in the block (the tube thermistor should be placed in position A2).

When you receive your unit, the thermistor is mounted in a HB-TC-3372 tube for 0.2ml blocks or a HB-TC-3505 tube for 0.5ml blocks.

NB: Do not disconnect a tube thermistor when a program utilising tube control is in progress. If this does occur, the program will be abandoned and an error message will be displayed.

NB: Tube thermistors from TouchDown, OmniGene and Omn-E instruments are not compatible and CANNOT be used with PCR Express thermal cyclers.

Extension Lead for the Tube Thermistor

The PCR Express is provided with an extension lead for the thermistor. This should only be used for transferring protocols from instruments that cannot use Active Tube Control. See Section 7.1 for further details.

Simulated Tube Control (SIM TUBE)

This temperature control method uses an algorithm similar to tube control. However, with simulated tube control, the block temperature overheat characteristic which is used to eliminate the sample temperature lag is based on calculated values, rather than the temperature monitored by the tube thermistor.

Similar considerations apply when transferring protocols from a block control machine as discussed above, and the extension lead can again be used.

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Reactions using Simulated Tube Control do not require the tube thermistor to be connected, but sample loading and volume details must be entered when prompted by the run screen. For all tube reactions in a 0.2ml or a 0.5ml thin walled tube, the volume factor is the **total reaction volume in µl** in one well, including any oil overlay. (*See Table 6.1*) For reactions in thick walled 0.5ml tubes, use (volume + 50) µl as a good approximation to achieving the same profile.

Table 6.1: Guidelines for Selecting Temperature Control Method and Entering Volume Factors

	Control Method	Loading Factor	Volume Factor	
Consumable Type			**0.5ml Block**	**0.2ml Block**
OmniPlate 96 well polycarbonate plate	SIM PLATE	No. of samples 1-96	vol (µl) x 10	vol (µl)
0.5ml 96 well plate	SIM PLATE	No. of samples 1-96	vol (µl)	-
0.5ml thin walled	SIM TUBE	No. of tubes 1-48	vol (µl)	-
0.5ml thick walled	SIM TUBE	No. of tubes 1-48	vol (µl) + 50	-
0.3ml thin walled	SIM TUBE	No. of tubes 1-96	vol (µl)	-
0.2ml thin walled	SIM TUBE	No. of tubes 1-96	-	vol (µl)
OmniTube 96 array	SIM TUBE	No. of tubes (96)	-	vol (µl)

Simulated Plate Control (SIM PLATE)

This mode of control operates on a similar principle to simulated tube control (*see Table 6.1*). However in this case, the control algorithm has been adjusted so that the programmed temperature is achieved in the volume inside a plate well. The system is optimised for the 0.2ml block and OmniPlate 96.

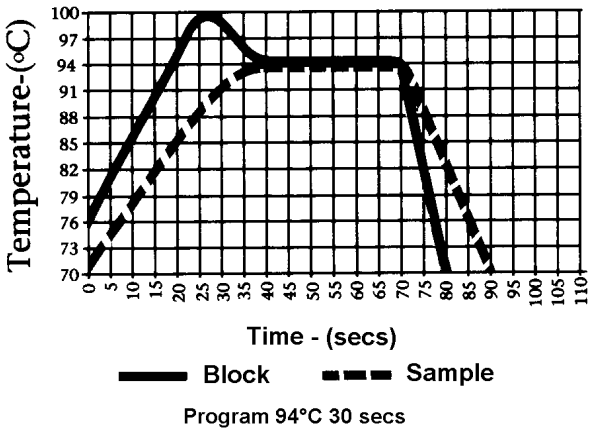
The use of 96 well plates on the 0.5ml block should be treated with caution. The OmniPlate 96 can and has been used successfully on the 0.5ml block. However, the fit is not optimal, and as a result there is a significant air gap between the plate and the block itself and the volume factor should be used to compensate. We recommend initially using a volume factor equal to 10 x reaction volume, but further optimisation may be necessary.

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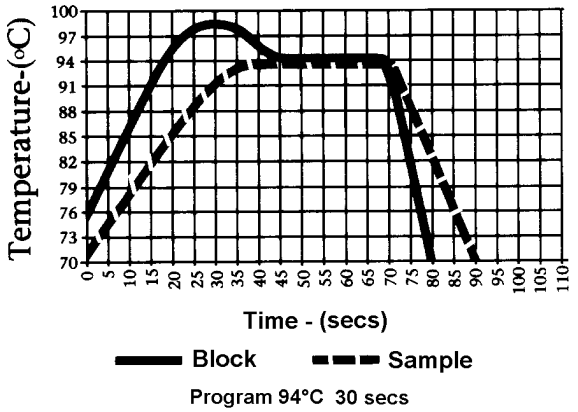
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Figure 6.2 Modes of Control used with the PCR Express

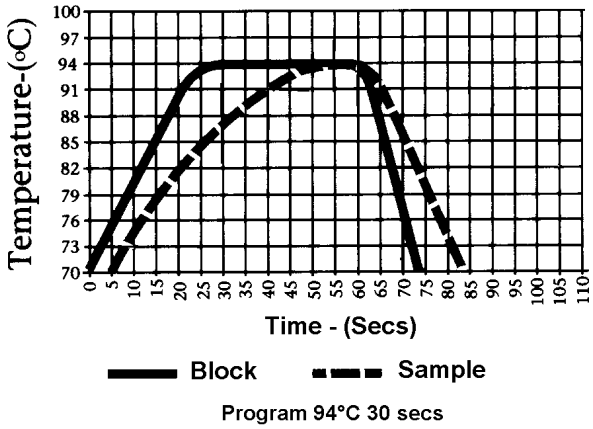
a. Active Tube Control



b. Simulated Control (Tube, Plate, Slide)



c. Block Control



Simulated Slide Control (SIM SLIDE)

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This mode of control operates on a similar principle to simulated tube control. However, in this case, the control algorithm has been adjusted so that the programmed temperature is achieved on the top surface of a standard microscope slide. The performance of the *In Situ* module has been measured using miniature thermistor probes attached to the surface of slides. Optimum results in terms of overshoot and uniformity across four standard glass microscope slides (0.8mm-1.0mm thickness) with the humidity chamber in place, have been obtained using a calibration factor of 200 (the default value). Using a higher calibration accelerates the approach to target temperature but results in some degree of overshoot. Conversely, a lower calibration factor slows the approach to target temperature.

Block Control

Controls block temperature as on a conventional dry block machine. We do not recommend this means of control for thermal cycling due to the variability in thermal profile obtained with different sample volumes and consumable types.

6.5 Heated Lid Preheat

Once a program and block have been selected, (and calibration factors entered where appropriate) the program will proceed according to the setup conditions as outlined below. The heated lid typically takes around 2 minutes to reach operating temperature. During this time, the block is controlled at 4°C.



WARNING: Both the tops of reaction vessels and the surfaces of the Heated Lid assembly (in particular the inner surfaces), can become very hot during normal operation. Touching the surfaces can cause burns. Do not touch the heated plate without safety gloves.

Heated Lid - Automatic Start

This should be selected for robust cycling reactions, where the reactants can withstand being incubated for the duration of the preheat (about 2 minutes) without the generation of spurious products. After the program is selected, the Lid Heating indicator light will switch on, denoting preheating of the lid. When the lid reaches operating temperature, the Ready/Working indicator light will change to green and the temperature cycling will start automatically. In this mode, samples should be loaded and the lid closed **before** starting the preheat.

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Heated Lid - Manual Start

This should be used for sensitive cycling reactions, where the reactants will not tolerate being incubated at low temperatures even for a short time, or where all risk of non-specific reactions must be eliminated. After the program is selected, the Lid Heating indicator light will switch on, denoting preheating of the lid. When the lid reaches operating temperature, the Ready/Working indicator light will change to green. An alarm will sound (if activated) and the **ENTER** key will need to be pressed to start temperature cycling. In this mode, samples should be mixed, loaded and the lid closed **after** the preheat is completed.

If, after 90 minutes, the **ENTER** key has not been pressed to start a program, the lid will switch off and the block will return to the idle condition.

No Heated Lid

If the heated lid is switched off (for example to perform experiments with oil overlay present), the temperature cycling will commence as soon as the program/control/calibration factors have been entered.

NB: The Lid Heating indicator light will remain off in this mode, and the Ready/Working light will come on immediately.

6.6. Hold & Pause Function

When entering a program the PCR Express will give you the opportunity to enter a 'Hold' temperature at the end of each stage. The samples will be held at this set temperature indefinitely. The screen will display HOLD during a run. If a "Hold" is inserted between stages, the program can be advanced to the next stage by pressing **CONTINUE**. If a "Hold" is inserted at the last stage, pressing **CONTINUE** will switch to the run summary screen.

Common uses of the Hold step include the following: -

- Inserting an initial 95^{o}C incubation at the start of the protocol to perform the 'Hot Start' procedure. After the enzyme has been added, pressing **CONTINUE** will advance the program into the cycling part of the protocol.
- A final low temperature (4-10^{o}C) hold for the end of overnight runs. Although unnecessary for the vast majority of protocols, some scientists prefer to have this step included.
- A final 72^{o}C incubation to ensure completion of the final extension step of a reaction.

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Pressing **PAUSE** during a cycling program will pause the program at the current or next target temperature within the step. Pressing **CONTINUE** will continue the countdown for the step.

6.7 Aborting Programs

The program will run to completion unless interrupted by the pressing of either the **PAUSE** or **STOP** keys whilst the appropriate run screen is displayed.

Pressing the **STOP** key once will display a verification screen (the program continues while this is displayed). Pressing **STOP** again aborts the program. Pressing **MENU** displays the Main Menu and continues the program.

6.8 Program Completion

At the end of the program the END OF RUN screen will be displayed. In this case, the heated lid will be switched off automatically.

The alarm (if selected) will sound and the block will remain at 20°C for 30 minutes to drive the heated lid plate temperature to ambient.

If a final HOLD temperature has been specified, the heated lid (if selected) will remain on.

For most thermal cycling applications a final HOLD at elevated temperature is not necessary. Extended high temperature hold steps can lead to evaporation and condensation problems, particularly with polycarbonate plates.

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CHAPTER 7
PCR EXPRESS THERMAL CYCLER
Troubleshooting

Check the volume in the tube thermistor

Check the volume of mineral oil in the control tube (a vial of suitable mineral oil is provided (alternatively Sigma molecular biology-grade mineral oil, M5904 or equivalent can be used). **Do not use aqueous solutions in the control tube.** Too much oil in the control tube will result in overshoots in the sample tube temperatures, whilst too little oil there will result in a time lag in the samples achieving temperature. The volume of oil in the control tube should match that in the reaction tubes, (including any oil overlay). Repeated overshoots at the denaturation temperature during temperature cycling, will reduce the activity of the thermostable enzyme resulting in poor yields of product. The correct position for the control tube is position A2.

Check location of thermistor within the control tube

Always ensure the thermistor probe is located centrally in the control tube and immersed in the liquid. If pushed against the side it will be measuring the temperature of the microcentrifuge tube and not the sample temperature.

Check the fit of the tube in the block

The PCR Express block accommodates most types of reaction tubes. The tube thermistor supplied with the PCR Express is mounted on an OmniTube, which should be changed periodically, as the fit of the tube will deteriorate with time. Reaction tubes should be distributed evenly in the block. If you are using a reaction tube that has not been recommended, check its fit in the PCR Express block before use. You should also remount the thermistor probe in this tube to ensure that your samples and the control tube are matched.

Check through the program

Before running a program, use the VIEW function to check that the correct combination of temperature and program number have been entered.

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Runs using Simulated Control Methods

1. Simulated Tube

Enter the number of samples, including the thermistor and the total reaction volume per tube including any oil overlay. 50µl should be added to the volume factor if thick walled tubes are used.

Use a setting of 96 samples and the correct reaction volume including oil overlay if a polypropylene OmniTube 96 is used.

2. Simulated Plate

0.2ml blocks

For 96 well microplates, (including OmniPlate 96), on a 0.2ml block, the volume factor entered is the individual sample volume. (NB: Polypropylene OmniTube 96 should be run under Sim Tube Control).

0.5ml blocks

For OmniPlates on a 0.5ml block, the volume factor entered is the total sample volume multiplied by 10 (to a maximum of 500). This accommodates the non-optimal fit of the OmniPlate 96 in the 0.5ml block. Other 96 well plates, for example the Costar ThermowellH, may be used with lower multiples of volume, but these need to be individually determined.

384 well blocks

For 384 well blocks the individual well volume should be used for the volume factor

3. Simulated Slide

For 0.8mm - 1mm thick glass slides using simulated slide control, the calibration factor is 200 (default value), other thicknesses will need optimization.

Check maximum and minimum values achieved during the run. This will give an indication of unusual temperature performance.

Power failure during a run

A message will appear on the main menu screen. The machine will either restart automatically when power is restored, or the program will be abandoned, as specified by the user on the SET

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UP menu screen. Viewing the run screens for the block will indicate the time of the power failure, and where applicable the time the run resumed.

7.1 Transfer of protocols from a block control machine to tube control using the thermistor lead extension

The different modes of control should be considered before transferring protocols directly. E.g., consider a temperature cycling protocol consisting of 1 minute at 95°C followed by 1 minute at 65°C, repeated 30 times. Using block control, the actual sample temperature is at 95°C for just 30 seconds at each step, a total of 15 minutes overall. In contrast tube control will result in precise one minute incubations at each step, a total of 30 minutes at the target denaturation temperature. Even though tube control gives a more accurate representation of the program, transferring such a protocol directly could result in lower yields because the enzyme is exposed to the high temperature for significantly longer, thus reducing its activity in later cycles.

The most accurate way to transfer protocols is to use the control tube of the PCR Express as a temperature probe in the block control machine as follows:

1. Set the PCR Express to run a single program at a set temperature for an extended time. (e.g. 37°C for 4 hrs), ensuring that the heated lid is switched **off** and the instrument is set to **block control**. This enables the tube thermistor to be used remotely.

2. Connect the thermistor lead extension to the thermistor control tube and plug it into the PCR Express.

3. Place the thermistor tube probe into the corresponding thermistor probe socket of the PCR Express, and locate the tube in a well of the block control machine running the required protocol.

4. After a short equilibration interval, the display on the PCR Express will indicate the sample tube temperature, which should be noted at regular time intervals (e.g. 10 seconds, for a number of cycles.) The actual length of time spent at each of the denaturation, annealing and elongation stages should be recorded, as well as any temperature overshoot values where the maximum/minimum temperature exceeds the target temperature or undershoots where the target temperature is not actually reached.

5. The temperature profile that the samples in the block control machine actually achieve, rather than simply the block temperature, can be used to program your PCR Express.

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Optimisation of protocols

The capability of the PCR Express programming enables protocol optimizations to be performed very rapidly. Typically, temperature cycling protocols may consist of three distinct stages:

1. Denaturation at an elevated temperature (usually 90-95°C).

2. Annealing at a temperature dictated by the melting temperature (Tm) of the oligonucleotides.

3. Enzymatic activity at a temperature dictated by the optimum temperature of the thermostable enzyme being used.

These three steps are typically repeated for twenty to thirty cycles depending on the amount of starting template.

Denaturation

The denaturation step at each cycle must be sufficient to denature the target DNA completely, including G-C rich regions. However, the effect on the enzyme activity of repeated high temperature incubations should also be considered. An extended initial denaturation step, (3 minutes, 95°C, before enzyme addition) will denature complex high molecular weight DNA template, but for later cycles this should be reduced to a maximum of 30 seconds at 92-95°C. Optimisation of the denaturation step is the most critical factor when transferring a protocol from a block control machine to tube control.

Annealing

The annealing temperature depends on the size and nucleotide composition of the oligonucleotides used. In general it varies between 50°C and 70°C and as a rough guide should be 5°C below the Tm. This may be calculated approximately using the following formula:

$$Tm = 2 \times (A + T) + 4 \times (G + C)$$

A difference in the annealing temperature of as little as 1°C can affect the specificity of a reaction, it is therefore recommended that a range of temperatures is tested to optimise the annealing temperature for each primer and template combination.

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Extension

The extension temperature is largely dependent upon the optimum temperature of the enzyme chosen and is usually in the range 70-75°C (see data sheet from manufacturer). The time required depends on the length of product being synthesised.

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CHAPTER 8
PCR EXPRESS THERMAL CYCLER
Maintenance

8.1 General Cleaning

1. All surfaces of the PCR Express system and Heated Lid should be cleaned regularly with a soft cloth, hot water and a mild detergent.

2. It is important to thoroughly dry all surfaces after cleaning.

3. The PCR Express is not intended for use with aggressive chemicals and on no account should organic solvents be used to clean this equipment.

4. Dampened cotton buds can be used to remove dirt and debris from individual wells. The wells should be kept clean to maintain optimum heat transfer performance.

8.2 Decontamination

When ^{35}S labelled nucleotides are thermally cycled they break down into lower molecular weight forms which are highly volatile and can leach through the walls of tubes and microtitre plates thus contaminating the block and possibly the heater plate of the heated lid.

We therefore do not recommend the use of ^{35}S labels, as replacing a dangerously contaminated block is expensive.

If ^{35}S labels are used, we recommend the following to minimize contamination.

1. Use a mineral oil overlay in all reactions, even when the Heated Lid is used.

2. If using tubes, use only the thick walled variety.

3. Use the thermal cycler in a fume hood, to minimize air contamination.

4. If using microtitre plates, coat the under surface with a thin layer of mineral oil.

5. If radioactivity must be used, the thermal cycling block and Heated Lid surfaces can be decontaminated using a 10 % v/v solution of **Neutracon** (Decon Lab Ltd, Conway Street Hove, East Sussex BN3 3LY Tel: +44(0) 1273-739241, Fax: +44(0) 1273-722088) or **PCC-54** (Pierce Eurochemie B.V Holland). Complete decontamination is unlikely, but low level counts can be achieved by repeated application of a fresh solution of 10% v/v Neutracon to the "Hot" area.

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All Control Chassis, Block Module, and Heated Lid components coming into contact with radioactivity should be decontaminated before re-use or transportation. In the event of returning a contaminated item please contact Thermo Hybaid's Service Department so that the appropriate handling arrangements can be made.

Thermo Hybaid recommends that if radioisotopes are to be used, equipment must be located in a designated Radiation Area. Local Radiation Safety procedures must be followed at all times.

The use of ^{35}S labelled nucleotides is not covered under our warranty agreement and requires special service arrangements.

8.3 Protection to the User

The PCR Express has been designed with operation safety in mind. In the rare event of an instrument failure, three levels of protection are built in to ensure the unit "fails safe". First, the software sets normal operating ranges for the block and lid. Should this fail; electrical circuitry is in place to ensure that safe temperatures are not exceeded. In the unlikely event of this failing, thermal fuses are fitted to shut off the power supply to damaged components.

The unit is fitted with an internal lithium battery containing a hazardous substance. This should be replaced with the original type and disposed of with care.

8.4 Protection of the Instrument

Fuses

The PCR Express mains power inlet is fitted with two T6.3A fuses (20mm x 5mm). If necessary these may be replaced by a qualified person.

8.5 Tube Thermistor Care

The tube thermistor assembly ensures that the programmed temperature profile is accurately and reproducibly achieved within all sample tubes. As a 'sample mimic', it is important that the thermistor is as close a representation as possible to the biological samples. To maintain accuracy and longevity of the tube thermistor Thermo Hybaid recommends the following:

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1) Do change the tube thermistor annually. It may be necessary to change the thermistor more often under heavy use (e.g. frequent removal of the thermistor from the tube).

2) Do check that the mineral oil volume is the same as the reaction volume including any oil overlay.

3) Do check and replace the control tube if it is showing signs of thermal degradation.

4) Do use the same type of tube for the thermistor tube as for the biological samples.

5) Do make sure the sensor part of the thermistor probe is centrally located at the base of the tube and is fully covered by mineral oil.

6) Don't use aqueous solutions in the thermistor tube.

7) Don't remove the control tube from the cap assembly by pulling the wire; gently lever the tube from the cap.

8) Don't kink the thermistor wire or crush the sensor part of the thermistor probe end.

9) Don't remove the thermistor assembly from the instrument by pulling on the wire, pull from the plug.

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CHAPTER 9
PCR EXPRESS THERMAL CYCLER
Technical Specifications & Ordering Information

Description	**PCR Express with 0.5ml Block**	**PCR Express with 0.2ml Block**
Catalogue Number	HB-PX-05-220/110	HB-PX-02-220/110
Block Capacity	48 x 0.5ml tubes	96 x 0.2ml tubes
	96 x 0.3ml tubes	1 OmniPlate 96
	1 x 96 well plate	
Temperature Control Available	Tube control	Tube control
	Simulated tube control	Simulated tube control
	Simulated plate control	Simulated plate control
	Block control	Block control
Performance		
Block Temperature Range	4°C - 99°C	4°C - 99°C
Block Heating Rate	Up to 3°C/Sec	Up to 3°C/Sec
Block Cooling Rate	Up to 2°C/Sec	Up to 2°C/Sec
Precision Control	1°C	1°C
Block Uniformity	± 0.4°C within 15 secs	± 0.4°C within 15 secs
Heated Lid Temperature Range	95°C - 120°C	95°C - 120°C
Display Resolution	0.1°C	0.1°C
Standard Accessories	Tube Thermistor 0.5ml	Tube Thermistor 0.2ml
	(HB-PX-TTM05)	(HB-PX-TTM02)
Interchangeable Block Modules	HB-PX-B05	HB-PX-B02
PCR Express Chassis	HB-PX-220/110	

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Description	**PCR Express with In Situ Block**	**PCR Express with 384 Well Block**
Catalogue Number	HB-PX-FB-220/110	HB-PX-384-220/110
Block Capacity	4 x microscope slides (76 x 26 x 1mm)	1 x OmniPlate 384
Temperature Control Available	Simulated slide control Block control	Simulated plate control Block control
Performance		
Block Temperature Range	4°C - 99°C	4°C - 99°C
Block Heating Rate	Up to 3°C/Sec	Up to 3°C/Sec
Block Cooling Rate	Up to 2°C/Sec	Up to 2°C/Sec
Precision Control	1°C	1°C
Block Uniformity	± 0.4°C within 15 secs	± 0.4°C within 15 secs
Heated Lid Temperature Range	95°C - 120°C*	95°C - 120°C
Display Resolution	0.1°C	0.1°C
Standard Accessories	Humidity Chamber	
Interchangeable Block Modules	HB-PX-BFB	HB-PX-B384

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PCR Express Gradient Cyclers

Description	**PCR Express with 0.5ml Gradient Block**	**PCR Express with 0.2ml Gradient Block**
Catalogue Number	HB-PX-G05-220/110	HB-PX-G02-220/110
Block Capacity	48 x 0.5ml tubes	96 x 0.2ml tubes
	96 x 0.3ml tubes	1 OmniPlate 96
	1 x 96 well plate	
Temperature Control Available	Tube control	Tube control
	Simulated tube control	Simulated tube control
	Simulated plate control	Simulated plate control
	Block control	Block control
Performance		
Block Temperature Range	4°C - 99°C	4°C - 99°C
Block Heating Rate	Up to 3°C/Sec	Up to 3°C/Sec
Block Cooling Rate	Up to 2°C/Sec	Up to 2°C/Sec
Precision Control	1°C	1°C
Block Uniformity	$\pm$ 0.4°C within 15 secs	$\pm$ 0.4°C within 15 secs
Heated Lid Temperature Range	95°C - 120°C	95°C - 120°C
Display Resolution	0.1°C	0.1°C
Standard Accessories	Tube Thermistor 0.5ml	Tube Thermistor 0.2ml
	(HB-PX-TTM05)	(HB-PX-TTM02)
Interchangeable Block Modules	HB-PX-BG05	HB-PX-BG02
PCR Express Chassis	HB-PX-220/110	

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PROGRAMMING (all unit variants)

Number of programs	99
Number of directories	6
Maximum number of program stages	10
Maximum number of steps per stage	10
Maximum programmed dwell time	9hr 59 mins 59 secs
Time increment/decrement	Yes
Temp increment/decrement	Yes
Temperature ramping	Yes
Pause facility	Yes
Autostart facility	Yes
Run “end time” calculations	Yes
File protection	Yes
Output	RS232
Alphanumeric programming	Yes
Gradient Software	Yes
Power	550W
Dimensions (WxDxH)	240mm x 390mm x 250mm
Weight	8.9kg

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Working Conditions

Ambient temperatures of 4°C to 35°C

Power requirements: 550W at 115/230V a.c. ± 10% and 50/60Hz.

Satisfies the requirements of BS EN 61010-1:1993.

NB: When the PCR Express temperature cycler is removed from a cold room, it should be left to equilibrate for 2-3 hours to avoid condensation.

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9.1 Consumables

Consumables	Pack Size	Description	Catalogue Number
Sealing Systems			
TD Tape	50	Sealing tape for polycarbonate plates	HB-TD-TAPE50
TDX Tape	100	Sealing tape for polypropylene plates	HB-TD-TAPE100
Compression Pad	1	Foam pressure pad for use with tape sealing system	HB-TD-SFOAM
OmniSeal TD Mat	5	Reusable 96 well sealing mat	HB-TD-MTSRS5

Plastic Ware

Consumables	Pack Size	Description	Catalogue Number
Plates			
OmniPlate 96	25	Polycarbonate 96 well plate	HB-TR3-MT
OmniPlate 96 Lid	25	Polycarbonate lids	HB-TR3-MTL
OmniFast 96	25	Polypropylene 96 well plate	HB-TC-8002N
OmniFast 384	50	Polypropylene 384 well plate	HB-TC-3840N
OmniTube 96	25	Single piece polypropylene plate	HB-TC-6002N
Tubes			
OmniTube 0.2ml	1000	0.2ml individual tubes with integral domed caps	HB-TC-3372N
OmniTube 0.2ml	1000	0.2ml individual tubes with integral flat caps	HB-TC-6202N
OmniTube 0.5ml	1000	0.5ml individual tubes with integral domed caps	HB-TC-4895N
OmniTube 0.5ml	1000	0.5ml individual tubes with integral flat caps	HB-TC-3505N
OmniStrip 0.2ml	2000	0.2ml strips of 8 tubes & caps	HB-TC-2662N
OmniStrip 0.3ml	2000	0.3ml strips of 8 tubes & caps	HB-TC-4043N
Racked OmniTubes	25	For 0.3ml 96 well plates (caps not included)	HB-TC-4073N
OmniTube Caps	2400	For all 0.2ml and 0.3ml OmniTube products	HB-TC-6022N

We recommend that domed cap tubes are used when the temperature control is set to Active Tube and flat cap tubes are used under Simulated Tube Control.

Polycarbonate can be affected by contact with formamide, DMSO and other organic solvents; these should therefore be avoided when using the polycarbonate OmniPlate 96.

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APPENDIX I
PCR EXPRESS THERMAL CYCLER
Software Upgrade via RS232

Thermo Hybaid issues software upgrades on a regular basis as new programming features become available. So that you can benefit from future innovations, the PCR Express software can be upgraded via an RS232 connection to the serial point of the PC using the cable provided.

Software is distributed free via Thermo Hybaid's Internet site (http://www.hybaid.com), or can be obtained on disk (PC) from your local Thermo Hybaid representative.

To upgrade the software you will need:

- A PC with a free COM1 port (9 way connector).
- Communications cable (supplied with your PCR Express).
- The PCR Express to be upgraded.

Upgrade Instructions (Dos)

- Ensure that the PCR Express is switched off.
- Connect the PC to the PCR Express using the cable supplied. One end should be connected to the COM1 port of the PC; the other should be connected to the 9-way connector on the underside of the PCR Express (next to the fan).
- Whilst simultaneously depressing the PAUSE and CONTINUE keys, switch on the PCR Express which will respond with three short bleeps and display a "READY TO LOAD SOFTWARE" message. The PAUSE and CONTINUE keys may then be released.
- On the PC, at the DOS prompt, type:
- A:UPLOADSA A:PCRE0200.BIN
- The PC will display the status of the upload as it progresses.
- When the upload is complete, the PC will display the message. Your PCR Express is now ready to use. Switch off the PCR Express and then switch back on in the normal manner (do not depress the PAUSE and CONTINUE keys).

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Upgrade Instructions (Windows 3.1)

- Insert Setup Disk 1 in drive A or Drive B.
- In Program Manager, click on **File** on the menu bar, click on **Run…**
- Type in the command line: **a:\setup.exe** if you inserted Setup Disk 1 in drive A, or type **b:\setup.exe** if you inserted Setup Disk 1 in drive B. Click the **OK** button.
- Follow the on-screen instructions given by the installation program.
- To upgrade your PCR-Express, double click the **Pcrexp** icon in the Windows Program Manager. Follow the on-screen instructions.

Upgrade Instructions (Windows 95)

- Insert Setup Disk 1 in drive A or Drive B.
- Click on **Start** and then **Run…**
- Type in the command line: **a:\setup.exe** if you inserted Setup Disk 1 in drive A, or type **b:\setup.exe** if you inserted Setup Disk 1 in drive B. Click the **OK** button.
- Follow the on-screen instructions given by the installation program.
- To upgrade your PCR-Express, click **Start**, select **Programs**, point to the **Pcrexp** folder and then click **Pcrexp**. Follow the on-screen instructions.

Upgrade Instructions (Internet)

Instructions will be given on Thermo Hybaid's Internet site.

It is important that the self test is not bypassed when first powering up a machine containing new software.

Verify that the self test completes correctly and that the correct software version is displayed on the SETUP MENU screen. The software upgrade process is now complete.

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APPENDIX II
PCR EXPRESS THERMAL CYCLER
Sealing Systems: The OmniSeal TD Mat & TD Tape

Overview

Thermo Hybaid offers two sealing systems to suit individual preferences. The TD Tape is a single use adhesive tape suitable for use with 96 well and 384 well systems, whereas the OmniSeal TD Mat is a re-usable silicone rubber mat designed for 96 well systems only.

How to use the TD/TDX Tape

TD Tape and **TDX Tape** are unique single use sealing systems for oil free thermal cycling in 96 well and 384 well plates which prevents cross contamination between wells. After a thermal cycling reaction TD or TDX tape can be removed from the plate with ease.

TD Tape

Green in colour and designed for use with **polycarbonate** plates such as OmniPlate 96.

TDX Tape

Clear in colour and designed for use with **polypropylene** plates such as OmniTube 96, OmniFast 96 or OmniFast 384.

TD and TDX tape cannot be interchanged, as the TDX tape will bind irreversibly to polycarbonate.

To secure contact with the heated lid of the thermal cycler the TD and TDX tapes must be used in combination with the reusable TD Compression Pad.

Sealing a 96/384 Well Plate

1. The tape can be applied to 96/384 well plates by positioning over one of the short edges and peeling back the lining material, allowing the adhesive layer to fall on to the plate. This should be possible without introducing creases. However, before heating, the adhesive is soft enough to be repositioned without adverse effects.

2. The tape should be firmly anchored by pressing over each well. This can be done using a soft roller or by finger pressure.

3. Once in the machine, the Compression Pad (HB-TD-SFOAM) must be placed on top of the sealed plate. The Compression Pad performs a dual function. It ensures that the tops

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of the sealed well reach the correct temperature and under compression it ensures the sealing between wells is intact.

Adding the TD Tape

Place a sealed 96 well/384 well plate on the machine. Select the "Plates" setting and close the lid.

Removing The TD Tape

Prior to removing the TD Tape/TDX Tape from a plate, ensure there is no condensation of the solution inside the wells or on the plugs of the tape by either:

1. Removing the 96 well plate from the block and cooling to 4°C.

2. Cooling down the PCR block to 4°C (if possible).

3. Heating the sample to 35°C for 30 minutes.

This will minimise the risk of well-to-well contamination when removing the TD Tape from the 96 well plate.

The TD Tape is most easily removed while the 96 well plate is in a rack or still on the machine. The sheet should be peeled back from one corner diagonally across the plate using a single smooth action. The TD Tape is easily removed and therefore is especially suitable for applications which require additions of reagents part way through the reaction, for example when performing reverse transcription and amplification in two stages.

Resealing Using TD Tape

TD Tape is designed for single use. Once heated and compressed, the backing conforms to the plate and cannot be easily repositioned. Re-use is therefore not advised.

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OmniSeal TD Mat

Cleaning & Sterilisation Procedures

The OmniSeal TD Mat (Catalogue N°: HB-TD-MT-SRS-5) is used to seal 96 well plates when using the PCR Express Heated Lid. Mats can be cleaned and sterilised using the procedures described below.

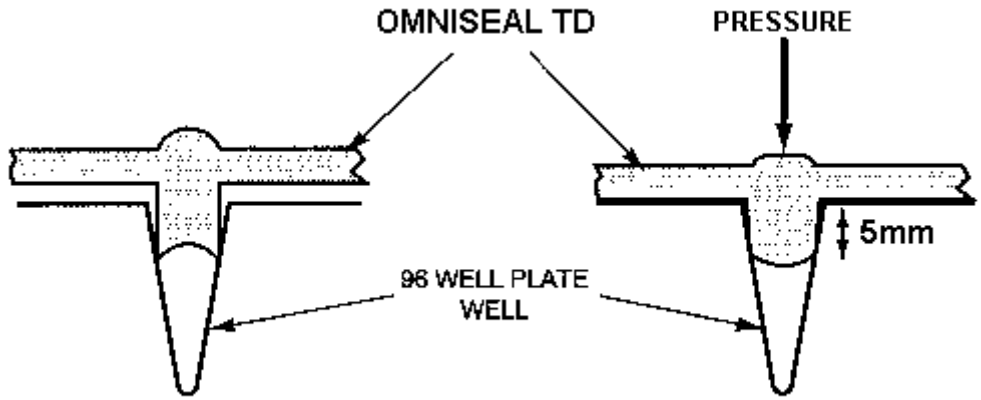
Soak for 1 minute in either 10% hypochlorite solution, or 0.1M hydrochloric acid solution and then rinse with distilled water. Dry inside an oven at up to 80°C if required. Autoclave at 121°C for 15 minutes.

Sealing a 96 Well Plate

OmniSeal TD Mats have 96 plugs in the underside. They are spaced so that they fit into the wells of a 96 well plate.

The plugs are shaped to expand sideways in order to seal all 96 wells of a 96 well plate, when sufficient pressure is exerted to the upper surface of the mat by the PCR Express Heated Lid.

Figure 10.1: *Showing how the OmniSeal TD Mat seals the wells of a 96 well plate.*



Removing the OmniSeal TD Mat

To minimise the risk of well-to-well contamination, remove the OmniSeal TD Mat from the 96 well plate whilst the plate is in a rack or on the machine.

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Re-usability of the OmniSeal TD Mats

The OmniSeal TD Mat can be re-used at least 5 times. Repeated washing and autoclaving of the Mats causes them to crack around the edges. When this starts to happen, they should be discarded.

NB: The number of times an OmniSeal TD Mat can be re-used is reduced considerably if it is cleaned with alcohols or detergents, or if exposed to UV irradiation.

Limitation of Microtitre Plate Operations

Some users have reported loss of volume when using microtitre plates for thermal cycling. Recent experiments performed at Thermo Hybaid have demonstrated that volume loss is independent of sample volume and occurs through the sides of the polycarbonate microtitre plate (all manufacturer plates tested) and is not due to poor sealing. Typically volume loss of 3µl per well in a 30-cycle reaction can be expected.

If you are working with small sample volumes it may be advisable to switch to polypropylene consumables instead.

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APPENDIX III

PCR EXPRESS THERMAL CYCLER

In Situ Block Application Notes

Overview

The PCR Express *In Situ* module with flat block has been designed for the precise control of temperature on up to four standard microscope slides (dimensions 76mm x 26mm x 1mm). Flat blocks are supplied with a Humidity Chamber, which can be used to reduce sample drying.

Temperature Control

The *In Situ* block can perform all the control functions of the standard PCR Express reaction tube block with the exceptions of Active Tube Control and Simulated Tube Control. Instead, the **Simulated Slide Control** option is used which minimises the time lag between the block reaching target temperature and the sample on the surface of the slide achieving target temperature. At the start of running a program on an *In Situ* module the **SIM SLIDE** option should be selected as the control mode (see *Figure 5.1*). The Heated Lid is not operational under this control mode.

The performance of the *In Situ* module has been measured using miniature thermistor probes attached to the surface of slides. Optimum results across four standard slides (1mm thickness) with the humidity chamber in place have been obtained using a **calibration factor** of 200. Using a higher calibration factor accelerates the approach to target temperature, but results in a degree of overshoot. Conversely, a lower calibration factor slows the approach to target temperature, but minimises any overshoot. A calibration factor of zero would be equivalent to block control when using **SIM SLIDE**.

Protocol Transfer

When transferring protocols from a conventional 'block control' machine, it is important to consider the effect of using PCR Express's 'Simulated Slide' control. On conventional thermal cyclers, the programmed temperatures commonly refer to the temperature achieved by the block and not the microscope slide. Consequently, a sample on the top surface of a glass slide will be significantly cooler than the block temperature. The PCR Express's 'Simulated Slide' control overcomes this problem by using an algorithm to ensure the temperature achieved by samples on the surface of the slides is as close as possible to the programmed temperature.

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In practice this difference in achieved temperature between different control systems is most pronounced at the denaturation step. As a guide, we recommend that when transferring protocols from a conventional thermal cycler to the PCR Express *In Situ*, the denaturation temperature is reduced by 3°C.

Programming

Programming and operation of the *In Situ* module are identical to the standard PCR Express tube modules (see Chapter 5) with the exceptions previously noted and also no heated lid operation is supported. For simple fixed temperature incubations of the slides, manual control can be used (Chapter 4). For more complex multi-step incubations or for temperature cycling reactions use Programmed Operation (Chapter 6).

Humidity Chamber

The polycarbonate Humidity Chamber has been designed to reduce drying out of the sample on the slides. For accurate temperature performance, the *In Situ* module should be used with the Humidity Chamber in place and the lid closed.

- Fill the reservoirs with water.
- Pre-heat the block with the Humidity Chamber in place. This allows the reservoir to warm up so that evaporation of the solution can begin. Alternatively, preheat the reservoir solution to the incubation temperature required.
- For thermal cycling with extended/high temperature incubations, it is important to seal the reagents under the cover slip. Thermo Hybaid recommends the use of EasiSeal sealing frames. These are available in three sizes (25µl - HB-OS-SSEZ1E, 65µl - HB-OS-SSEZ2E, and 125µl - HB-OS-SSEZ3E). They will completely seal the specimen area to prevent drying.

NB: If a petroleum based rubber compound is used for sealing cover slips in place, the compound should be allowed to dry out completely before use with this system. If complete drying is not allowed, the solvent vapour will damage the surface of the Humidity Chamber.

More information on the use of In Situ applications using Thermo Hybaid equipment can be obtained from the Thermo Hybaid In Situ Hybridization Guide, which is available free upon request.

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APPENDIX IV

PCR EXPRESS THERMAL CYCLER

Comparative Performance Data between Thermo Hybaid Ambient & Sub-Ambient Block Systems

Although there are fundamental differences between the methods of heating and cooling for these different block systems, in the majority of cases, direct transfer of protocols will be possible. This is because the temperature and time control system is similar for the PCR Express, TouchDown, Omn-E and OmniGene systems, even though the performance and accuracy characteristics are different. There are some cases however, when the biological performance of the two instruments for a given cycling protocol will be different. One obvious example is for reactions with annealing temperatures close to ambient. Less obvious is the effect of default ramp rates on other stages of the reaction (see Table 7.1).

Table 7.1: *Block Ramping Characteristics of Ambient and Sub-ambient Units*

CYCLING STAGE	AMBIENT	SUB-AMBIENT	NOTES
Extension-Denaturation	Essentially linear to within 5°C of setpoint. (Max. up to 2°C/second)	Essentially linear to within 5°C of setpoint. (Max. up to 3°C/second)	Ambient denaturation setpoint times may be able to be decreased (e.g. 5 sec/cycle) compared to sub-ambient because of the latter's faster approach to setpoint
Denaturation-Annealing	Faster the further away from ambient. (Max. up to 2°C/second (90-80°C)- Effect of decrease minimal in operating range to ambient+10°C)	Essentially linear to within 5°C of setpoint for settings down to 20°C. (Max. up to 1.8°C/second)	Fundamentally, the sub-ambient unit will give better temperature control for newer applications requiring annealing temperatures close to ambient
Annealing-Extension	Essentially linear to within 5°C of setpoint. (Max. up to 2°C/second)	Essentially linear to within 5°C of setpoint. (Max. up to 3°C/second)	The more rapid heating rate of the sub-ambient units from the annealing temperature may need to be slowed using ramp rate control to mimic the temperature profile of a reaction optimised on an ambient version

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APPENDIX V

PCR EXPRESS THERMAL CYCLER

Glossary of Terms

Advanced Edit	Allows the adjustment of the following: 1) Incrementation/decrementation of time and/or temperature on a cycle-by-cycle basis. 2) Control of temperature ramping between set points.
Block Control	The temperature of the cycling block is monitored and controlled by the thermistor mounted on the block. **Because of the time lag between the block and sample reaching temperature we recommend that tube control is used.**
Block Module	The interchangeable assembly comprising sample block, Peltier array, heatsink and control thermistor.
Control Chassis	The main body of the machine, incorporating keypad, control and power functions, fan and display. The Heated Lid is fitted to the control chassis.
Copy	Copy is used to make identical copies of stored programs, prior to editing.
Edit	Edit is used to create new programs or to change existing programs.
Erase	Erase is used to delete programs or create extra space for new protocols.
GRADIENT CALCULATOR Software	Software for use in calculating optimized annealing temperatures after gradient cycling.
HOLD	A HOLD step may be specified at the end of a stage. Samples are held at this temperature until **CONTINUE** is pressed to resume the program. This function can be used to hold samples at a fixed temperature while a reagent is added before temperature cycling starts.
In Situ Module	The PCR Express Flat Block, for performing *In Situ* reactions on up to four microscope slides.
Loading Factor	To compensate for differing numbers of samples, enter the number of tubes for each cycling run.
MAN Manual Operation	Manual operation is used for single temperature incubations under block control. The incubation will proceed at the set temperature until the **STOP** button is pressed; or until a new temperature is specified.
Menu	The menu key allows the user to return to the main menu at any time without making changes in the programming/operation of the unit.

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OPTS	Allows the accessing of SETUP options or GRADIENT CALCULATOR Software
New Temperature	A new temperature may be specified during a manual incubation step. The manual program is interrupted and the sample temperature rapidly changes to the newly specified temperature.
Peltier Device	A thermoelectric device incorporating a bismuth telluride semi-conductor crystal array. On passing a current through the crystal, one surface of the crystal is heated, the other cooled. Reversing the current reverses the heat flow.
Power Failure	The PCR Express has the facility to restart after a mains power supply failure. There are two set up options for restarting after a power failure: 1. Resume program at step that power failure occurred. 2. Abandon program at power failure step. One of the above should be specified using the SETUP menu.
PROGRAM	Select PROG from the main menu to access the programming menu.
Ramp Rate (Temperature Ramping)	Ramping precisely controls the rate of change of sample temperature (ºC/sec). This is useful for limiting the rate of change of temperature, to allow partial extension of short or degenerate primers.
RUN	The RUN Option allows the user to run a stored program on the block
Sample Volume	When using simulated tube control methods, the sample is entered to ensure precise block heating and cooling.
SET UP	This function allows the default settings of the PCR Express (set day and time, power restart option and screen brightness) to be modified.
Simulated Plate Control	Simulated Plate Control incorporates an algorithm to reduce time to reach target temperature, using precise overshoots in block temperature for each setpoint. Refer to Section 5.2 for instructions on how to use this control option.
Simulated Slide Control	Simulated Slide Control incorporates an algorithm to reduce the time to target temperature when performing temperature cycling reactions using microscope slides on an *In Situ* block.
Simulated Tube Control	Simulated Tube Control incorporates an algorithm to reduce the time to reach target temperature, using precise overshoots in block temperature for each setpoint. The calculated sample temperature inside sample tubes and block temperature are displayed during the run.

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Stage	One stage of a program consists of one or more steps up to a maximum of 10. A stage may be repeated for temperature cycling. Up to ten stages may be used in one program, and stage-to-stage linking is automatic.
Step	A step consists of a programmed temperature and time interval. Time increment and ramp rate may also be specified if required. Enter zero for each heading to specify the last step of a particular stage, and enter zero for each heading at the first step of the final stage.
Sub-ambient Block	The aluminium block used on sub-ambient PCR Express systems. This uses Peltier devices for heating and cooling. Operating range: 4°C to 99°C.
TEMP INC/DEC Temperature Increment/Decrement	May be used to increase/decrease a temperature set point at successive cycles. E.g. a decrement of 0.1°C will give 60, 59.9, 59.8, 59.7.... °C. This is useful for touchdown PCR reactions where stringency at the annealing step is decreased as the reaction proceeds.
Thermistor	A thermistor is a resistor whose resistance changes with temperature. It can therefore be used as a very accurate temperature probe with small liquid volumes.
TIME INC/DEC Time Increment/Decrement	May be used to change a time interval to each repeat cycle of a specific step. E.g. an increment of 10 seconds on a 60 second step will give time intervals of 60, 70, 80, 90, 100....seconds. This is useful when increasing an incubation step to allow for the depletion of an enzyme.
Touchdown PCR	A gene amplification procedure where the annealing temperature is high (e.g. 65°C) to achieve high specificity at the start of the reaction. It can then be reduced with successive cycles (by e.g. 0.5°C/Cycle) to increase yield once high stringency is no longer required.
TUBE (Active) Tube Control	This type of software control is dependent upon the external thermistor probe located in the microfuge tube. This allows very accurate monitoring and control of sample tube temperature. This feedback control loop allows the transition to target temperature to be accelerated by creating a temperature gradient between the block temperature and the tube temperature.
HEATED LID	A standard fitting which allows oil free cycling in 0.2, 0.3, 0.5ml tubes and 96/384 well plates. The auto-height adjusting heated plate in contact with the top of the reaction vessels maintains the temperature above 100ºC, preventing evaporation of the sample.

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View	The View function allows the contents of programs to be accessed.

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APPENDIX VI

PCR EXPRESS THERMAL CYCLER

Pre-Set Programs on the PCR Express

Programs coded into the F:HYBAID Directory of the PCR Express memory can be used directly or as building blocks for other protocols using the COPY function.

PROGRAM F:90	**3T_PCR**			
Stage 1	Step 1	94°C	0:01:00	x 1
Stage 2	Step 1	94°C	0:00:30	
	Step 2	55°C	0:00:30	
	Step 3	72°C	0:00:30	x 30
Stage 3	Step 1	72°C	0:05:00	x 1
	Hold	4°C		
PROGRAM F:91	**2T_PCR**			
Stage 1	Step 1	94°C	0:01:00	x 1
Stage 2	Step 1	94°C	0:00:10	
	Step 2	68°C	0:00:10	x 30
Stage 3	Step 1	72°C	0:05:00	x 1
	Hold	4°C		
PROGRAM F:92	**LONGPCR**			
Stage 1	Step 1	94°C	0:02:00	x 1
Stage 2	Step 1	94°C	0:00:10	
	Step 2	65°C	0:00:30	
	Step 3	68°C	0:10:00	x 10
Stage 3	Step 1	94°C	0:00:10	
	Step 2	65°C	0:00:30	
	Step 3	68°C	0:10:00 + Time Inc. 0:20/cycle	x 20
Stage 4	Step 1	68°C	0:07:00	x 1

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PROGRAM F:93	**CYCSEQ**			
Stage 1	Step 1	94°C	0:01:00	x 1
Stage 2	Step 1	94°C	0:00:10	
	Step 2	50°C	0:00:10	
	Step 3	60°C	0:04:00	x 25
	Hold	10°C		
PROGRAM F:94	**TD_PCR**			
Stage 1	Step 1	94°C	0:01:00	x 1
Stage 2	Step 1	94°C	0:00:30	
	Step 2	65°C	0:00:30	
	Step 3	72°C	0:00:30	x 4
Stage 3	Step 1	94°C	0:00:30	
	Step 2	65°C	0:00:30 + Temp DEC-1.0°C /cycle	
	Step 3	72°C	0:00:30	x 16
Stage 4	Step 1	94°C	0:00:30	
	Step 2	50°C	0:00:30	
	Step 3	72°C	0:00:30	x 5
Stage 5	Step 1	72°C	0:05:00	x 1

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PROGRAM F:95	**RTPCR37**			
Stage 1	Step 1	37°C	1:00:00	
	Step 2	95°C	0:10:00	x 1
	Hold	4°C		
Stage 2	Step 1	95°C	0:00:30	
	Step 2	55°C	0:00:30	
	Step 3	72°C	0:00:30	x 40
Stage 3	Step 1	72°C	0:05:00	x 1
PROGRAM F:96	**RTPCR65**			
Stage 1	Step 1	65°C	1:00:00	
	Step 2	94°C	0:01:00	
Stage 2	Step 1	94°C	0:00:15	
	Step 2	65°C	0:00:30	x 40
Stage 3	Step 1	65°C	0:07:00	x 1
PROGRAM F:97	**RAMP**			
Stage 1	Step 1	94°C	0:01:00	x 1
Stage 2	Step 1	94°C	0:00:30	Ramp 1.0°C/Sec
	Step2	55°C	0:00:30	Ramp 1.0°C/Sec
	Step 3	72°C	0:00:30	Ramp 1.0°C/Sec
Stage 3	Step 1	72°C	0:05:00	x 1

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PROGRAM F:98	**DIGEST**			
Stage 1	Step 1	37°C	4:00:00	x 1
PROGRAM F:99	**CUT/KIL**			
Stage 1	Step 1	37°C	4:00:00	
	Step 2	95°C	0:15:00	x 1
	Hold	4°C		

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