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THERMO HYBAID

Px2 Thermal Cycler

USER INSTRUCTION MANUAL



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Px2 THERMAL CYCLER
PCR License Registration

Authorised Thermal Cycler

This instrument, Serial No. ..... is an Authorised Thermal Cycler. Its purchase price includes the up-front fee component of a license under United States Patent Nos. 4,683,195, 4,683,202 and 4,965,188, owned by Roche Molecular Systems, Inc., and under corresponding claims in patents outside the United States, owned by F. Hoffmann-La Roche Ltd, covering the Polymerase Chain Reaction ("PCR") process, to practise the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

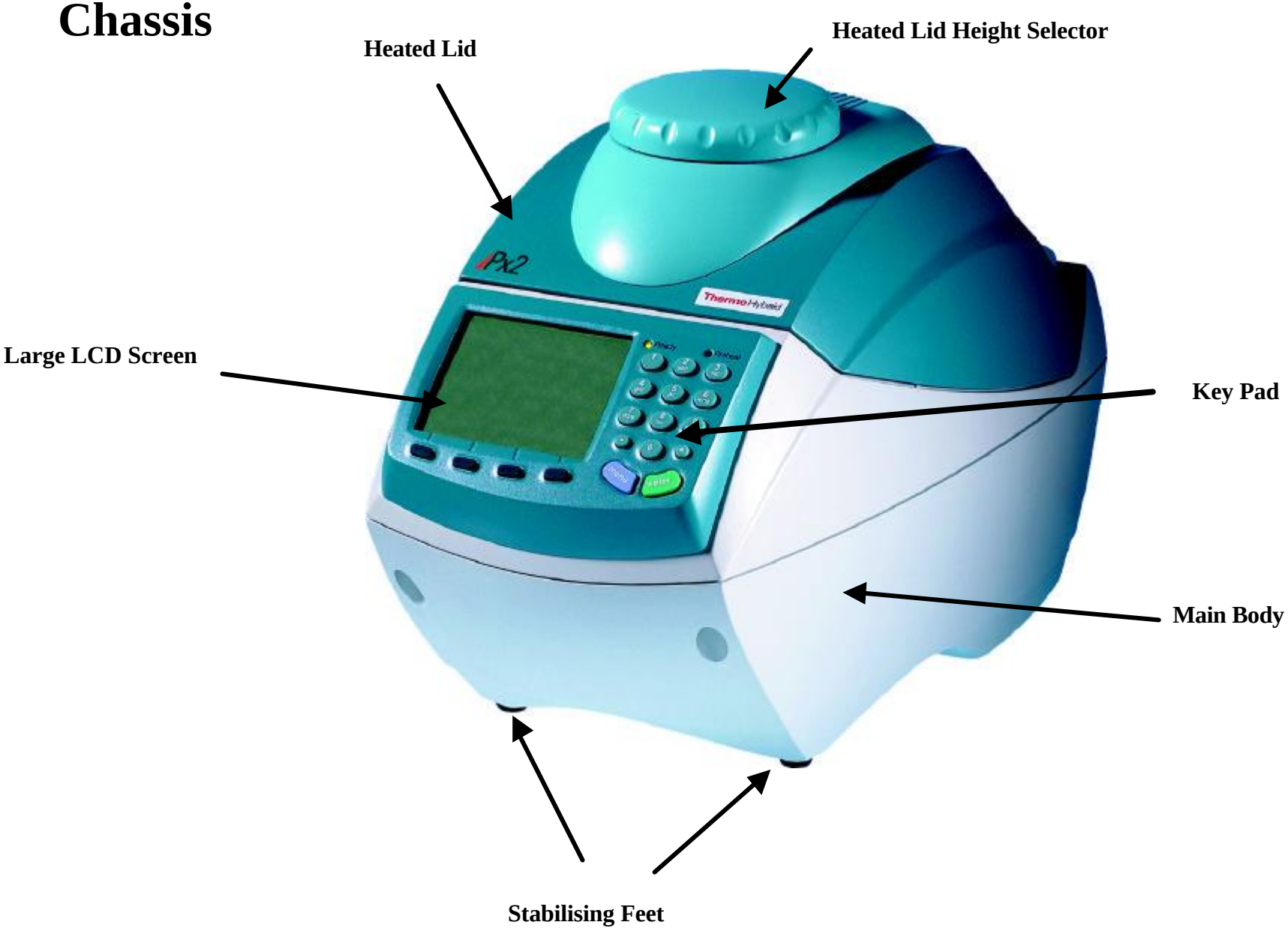
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Chassis



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Px2 THERMAL CYCLER
Warranty

Thermo Hybaid guarantees that the Px2 Thermal Cycler you have received has been thoroughly tested and meets its published specification.

This warranty is valid for 24 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 Px2 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 Px2 unit is guaranteed for 90 days.

Thermo Hybaid reserves the right to alter the specification of the Px2 without prior notice. This will enable us to implement developments as soon as they arise.

The Thermo Hybaid Px2 is for research use only.

Read the Instruction Manual carefully before using the Px2 to ensure that you obtain the best possible results from the machine.

NB: The Px2 should only be used by suitably qualified and trained people. If the Px2 is not used as specified in this Manual, the protection provided by the equipment may be impaired.

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CHAPTER 1
Px2 THERMAL CYCLER
Introduction

1.1 Overview

Px2 is the essence of fast, accurate licensed thermal cycling. Px2 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 Px2 System

The Px2 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 Standard and Gradient Block Module

This block can hold any of the following:

- 1 x OmniUtra 96 well skirted polycarbonate plate
- 1 x OmniFast 96 well skirted polypropylene plate
- 1 x OmniTube 96 single piece polypropylene plate
- 96 x 0.2ml OmniStrip tubes
- 96 x 0.2ml individual OmniTubes

The 0.5ml Standard and Gradient Block Module

This block can hold the following:

- 48 standard 0.5ml individual OmniTubes

The Flat Block Module

This block accommodates up to four standard microscope slides.

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The 384 Well Block Module

This block accommodates 1 x OmniFast 384 well skirted polypropylene plate.

Temperature Control Methods

The temperature cycling blocks for the system are specially coated precision-machined aluminium. 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. A 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 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 Px2 **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 RAPDs 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.

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Programming and Operation

The combination of new keypad design and large screen display ensure user friendly programming and operation. During programmed operation the display screens provide comprehensive information relating to your protocol including sample temperature, number of cycles completed, estimated time for completion, etc.

The Px2 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 option.

Pre-set Programs

The Px2 is supplied with ten (non-editable) pre-set protocols, stored as programs 90-99 in the F:THERMO directory, enabling users to run experiments immediately. These pre-set protocols cover the most common thermal cycling techniques, and can be used to create customised protocols by editing (see Appendix V).

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 Px2 cycling reactions where the annealing temperature is decreased with successive cycles.

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Advanced Edit – Gradient (optional)

For rapid optimisation of primer annealing parameters the Px2 gradient feature should be used. A maximum temperature gradient of 15°C can be programmed across the 96 well block.

Calibration of your Px2

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 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.

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 reducing condensation of the reaction mixture as it is repeatedly heated and cooled. Condensation on the walls of the reaction vessels below the level of the block is a feature of block based thermal cyclers. The level of this condensation is very small and will not affect the reaction in most cases. In very small volume reactions it may however be necessary to use an oil overlay.

Operating the Lid

Opening the Lid

The lid is opened by turning the knob so that the 'open lid' icon is aligned to the front of the unit (see Figure 1.1). This releases the catches to raise the lid and expose the block recess.

Closing the Lid

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To close, gently lower the lid. At this point a 'click' should be heard as the catches are engaged. This action lowers the heated lid onto the samples and closes the block, ensuring a tight contact.

The pressure; exerted by the lid can be adjusted for tubes or plates by rotating the knob so that the relevant icon is aligned to the front of the unit (see Figure 1.1). It is defaulted to the tube pressure, for the plate pressure turn the knob clockwise until the plate icon is aligned with the front of the unit. Careful consideration should be taken in selecting which setting should be used. When using a 0.2ml block, any consumable (including compression pad) whose height exceeds 10mm above the rim of the block should be used on tube setting whether it is a plate or not. With a 0.5ml blocks, any consumable (including compression pad) whose height exceeds 5mm above the rim of the block should be used on tube setting whether it is a plate or not. **Please note that the tube thermistor must be removed before selecting the plate mode.**



Figure 1.1: Open Lid Icon Tube Icon Plate Icon

The temperature of the heating plate is set at 120°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 2-3 minutes before cycling commences. During this heating time, the block is automatically cooled to 4°C to maintain sample integrity. The block can be operated with or without the lid being switched on (the lid is automatically deactivated in units fitted with flat block modules).

<u>WARNING:</u> 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.

1.4 Input / Output Sockets

- The Px2 has a power input socket located on the back of the unit

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- Communications to the unit may be facilitated by the RS232 / 485 Comm Port via 9 way D-Type connector. The communication protocol may be selected using the slide switch adjacent to the connector (located on the base of the unit). The position closet to the front of the unit corresponds to the RS232, whilst the position closest to the back of the unit corresponds to RS485.

1.5 Support Services

The Px2 has been designed for reliability and for ease of maintenance. Thermo Hybaid continues to offer full service and technical support for all its products.

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Tel: +44 (0) 1784 425 000

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CHAPTER 2
Px2 THERMAL CYCLER
Safety Precautions

2.1 General Safety Precautions

1. The Px2 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 Px2 is a class 1 appliance. To minimise 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 authorised 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 authorised 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 changing 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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2.2 Symbols & Conventions

The Px2 has been designed for safe operation. The following symbols appear 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
Px2 THERMAL CYCLER
Unpacking & Installation

3.1 Unpacking

Before unpacking the Px2, please make sure that the outer packaging is undamaged.

After unpacking, ensure all packaging and fixtures are retained. Should there ever be a requirement to move the unit, it 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.

N.B. If the unit is transported or stored in conditions of high humidity it must be allowed to stabilise at normal ambient temperature before powering up the unit.

3.2 Packing List

1. Px2 Control Chassis
2. Px2 Block Module (optional)
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

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

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 Px2 systems, excess heat is removed from the system by a fan. The air intake for the fan is located underneath the unit and air is expelled out of the back of the unit. To ensure correct airflow around the unit, allow at least 8cm clearance between the sides of each module and 15cm of clearance at the back of the unit. **Special 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 Px2 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 Px2 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.

Block Installation and Removal

1. Remove the Px2 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 turning the knob anticlockwise to the open lid icon) 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:

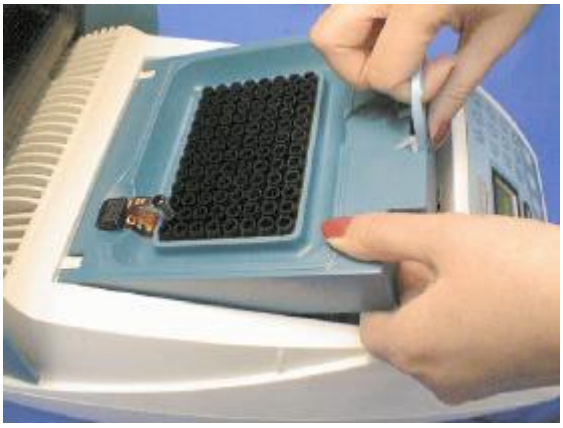
SELF CHECK FAILED	or	SELF CHECK FAILED
BLOCK NOT PRESENT		BLOCK ID ERROR

Removal is the reverse procedure.

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Figure 3.1 Installing the Block Module

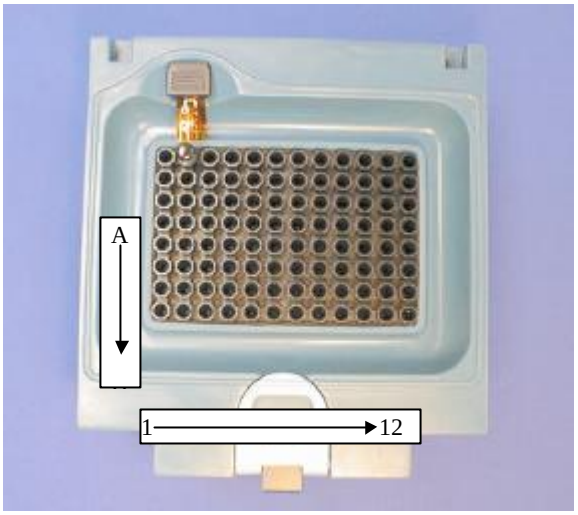


Thermistor Positioning

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 and not touching the wall of the reaction vessel.

Figure 3.2 Well Numbering Showing Tube Thermistor Positioning in A2



Use in Cold Rooms

The Px2 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
Px2 THERMAL CYCLER
Operating Instructions

4.1 Initial Start Up

(a) Switch on the Px2 using 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 5 seconds a screen will display "Self-check procedure in progress".

The self-test routine checks the block thermistor, heating and cooling circuits and the heated lid. This takes approximately 30 seconds. (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, 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 contrast settings and to identify software version fitted. Select **CALC** to access the gradient calculator software (only relevant to gradient protocols).

Selection of the alternative 'Main' Menu functions is achieved by depressing the soft key directly beneath the relevant display. These functions are discussed fully in the following chapters.

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4.3 Operating Keys

The Px2 software is designed to be intuitive; the following table gives a summary of the primary functions of each of the operating keys.

Table 4.2 Function 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).
ALPHANUMERIC KEYS	- Used for entering parameters - Used for selecting programs - Alphanumeric naming of programs/directories
SOFTKEYS	- **These keys alter function depending on the screen and the state of the block e.g. active or inactive** - Start/Stop programs - Navigate around the screen - Navigate between screens - Used to toggle between options

4.4 Set Up Functions

Selecting **OPTS** at the Main Menu by pressing the relevant soft key gives access to the **SETUP** menu.

Within the **SETUP** screen, information about the type of block fitted, the current software version installed, the time and the date are displayed.

A flashing cursor beneath **CLOCK** now indicates that this application will be selected when **ENTER** is pressed. The **LEFT** or **RIGHT** arrow keys can be used to alter the selection and a **BACK** button is available to return to the previous screen

Clock

The time and date format of the clock display can be modified. To set the clock or alter the format, select the **SETUP** screen followed by **CLOCK**

The American (MM/DD/YY) or European (DD/MM/YY) format can be selected using the **TOGGLE** softkey.

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Press **ENTER** or use the **PREVIOUS** and **NEXT** softkeys to move to the time/date setting fields. Use the number keys to enter time (hrs & mins) and day/month/year information. Press **BACK** to the previous screen or **ENTER** to confirm changes and return to the Main Menu.

Power Failure Resume

If there is a power interruption during a run, the Px2 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 **TOGGLE** softkey.

Press the **CONTINUE** softkey 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.)*

4.5 Gradient Calculation

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.

- To access the Gradient Calculator select **OPTS** from the MAIN MENU.
- Using the left and right navigation keys, move the cursor to **CALC** and press **ENTER**.
- The block type you are using will automatically be displayed. Enter the minimum annealing temperature and gradient range used in your optimisation experiment, using the alphanumeric keypad.
- The well temperatures are displayed in sequence in a bar chart formation from column 1 to 12 with the calculated temperature for each column displayed to the left of the bar.
- Press **BACK** to return to the Gradient Calculator screen to enter new parameters or alternatively press **MENU** to return to the Main Menu screen.

The Gradient Calculator may be accessed at any time, even when the block is active. MENU should be pressed to return to the Main Menu and the steps described above should be followed.

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4.6 Running a Program

Design of the Px2 software ensures that running a pre-stored protocol is easy.

- At the Main Menu, select **RUN** by depressing the relevant softkey.
- Select the source directory (e.g. F: THERMO) using the directional **UP** and **DOWN** softkeys and press **ENTER**. Select the program number to be run using the number pad, or scroll through the programs using the **PREVIOUS** and **NEXT** softkeys and press **ENTER** at the appropriate choice. (Please note the **UP** and **DOWN** keys may be used to advance or return to a parameter if necessary).
- Once the relevant protocol is selected the settings for heated lid operation, holdstep, loading and end run settings can be defined. The **PREVIOUS** and **NEXT** keys should be used to scroll between the various possibilities, whilst the **ENTER** key can be used to select the relevant option.
- Lastly, the temperature control method is selected by using the **PREVIOUS** and **NEXT** keys to change to the appropriate method. A **BACK** button can be used to return to the previous screen if necessary.
- Pressing **ENTER** will now start the program.

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.

4.7 Manual Operation

This option is used for static incubations when thermal cycling is not required, e.g. probe denaturations and enzyme reactions. In this mode the unit will only operate under block control (see Chapter 6 for details).

1. Select **MAN** from the Main Menu by depressing the relevant softkey.
2. Enter the name of the user or protocol if desired using the alphanumeric keys. Move to the next/previous character using the **LEFT** and **RIGHT** arrow softkeys. Pressing **ENTER** moves the cursor to the temperature selection (this occurs even if a user name has not been entered).
3. Enter the temperature using the alphanumeric keys. Press **ENTER** to continue.
4. Select the heated lid on (auto) or off using the **LEFT** and **RIGHT** softkeys.

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5. Press **ENTER** to start the incubation. The screen displays the target temperature and actual temperature of the block together with the elapsed time. The count-up timer will start when the block reaches temperature.

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 the **STOP** softkey followed by **ABORT** to cancel the program.

7. Press the **NEW TEMP** softkey to change the set temperature, and then **CONTINUE** to continue the program with a new temperature.

4.8 Programming Function

PCR protocols of varying degrees of complexity can easily be created using Px2. As with all operations of the Px2, programming is accessed from the Main Menu.

Access the Program Menu by selecting **PROG** from the main menu. If at any time you wish to stop programming, press **MENU** to return to the Main Menu. Four options are available for programming. The **LEFT** or **RIGHT** arrow keys are used to move the cursor to the required menu option. Press **ENTER** to then select that option:

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

   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 Px2.

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.

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4.9 Loading Samples and Heated Lid Operation

The Px2 will produce identical cycling profiles whether 1 or 96 samples are to be analysed.

The following guidelines maximise the uniformity and thermal transfer characteristics of the block thus ensuring optimal sample to sample reproducibility:

- Load samples uniformly across the block rather than in clusters. Spreading the samples spreads the thermal load and helps maintain 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 contact between the heated lid and the tops of the tubes, allowing good thermal transfer.
- Care should be taken when matching block, consumable, control mode and sample number (see Appendix I for a full list of recommended consumables).
- Ensure tube caps are properly closed before loading the block.

The 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.

The pressure exerted by the lid can be adjusted for tubes or plates by rotating the knob so that the relevant icon is aligned to the front of the unit (see Figure 4.1). It is defaulted to the tube pressure; for the plate pressure turn the knob clockwise until the plate icon is aligned with the front of the unit. Careful consideration should be taken in selecting which setting should be used. When using a 0.2ml block, any consumable (including compression pad) whose height exceeds 10mm above the rim of the block should be used on tube setting whether it is a plate or not. When using a 0.5ml block, any consumable (including compression pad) whose height exceeds 5mm above the rim of the block should be used on tube setting whether it is a plate or not. **Please note that the tube thermistor must be removed before selecting the plate mode.**



Figure 4.1: Open Lid Icon Tube Icon Plate Icon

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CHAPTER 5
Px2 THERMAL CYCLER
Programming the *Px2*

5.1 Introduction

The Px2 has been designed to make programming user friendly and easy. The memory will hold up to 99 complete thermal cycling protocols including 10 pre-programmed template programs (Nos. 90-99 detailed in Appendix V). Programs are identified by a number (01-99) and a user defined name (up to 7 characters). Each program protocol type may be assigned to a directory, enabling you to group programs by user name.

To enter a new program you simply

i) Enter or 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** using the appropriate softkey.

2. With the cursor highlighting **EDIT,** press **ENTER.**

3. Use the **LEFT** and **RIGHT** navigation key to highlight **NEW** with the cursor and press **ENTER**.

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4. Use the **LEFT** and **RIGHT** navigation key to highlight **NEW DIR** with the cursor and press **ENTER.**

5. Select the desired target directory (A-E) using the **UP** and **DOWN** softkeys and then **ENTER**.

6. You are now able to name the directory using the alphanumeric keypad. Use the **PREVIOUS** and **NEXT** softkeys to move to the position of the cursor where the character is to be added. Press **ENTER** only when the name is complete. If no characters are entered, the directory name will remain blank. The **BACK** button may be used to return to the previous screen if necessary.

Editing a Directory Name

To change the name of an existing directory simply retype the new directory name over the old one.

1. Select **PROG** from the Main Menu using the appropriate softkey.
2. The cursor should be flashing below **EDIT**, select this option by pressing **ENTER.**
3. Use the **RIGHT** softkey to move the cursor to **NEW** and select by pressing **ENTER.**
4. Use the **RIGHT** softkey to move the cursor to **NEW DIR** and select by pressing **ENTER.**
5. Use the **UP** and **DOWN** cursor to select the directory you wish to rename and press **ENTER.**
6. Use the alphanumeric keypad to enter the new directory name. Use the **PREVIOUS** and **NEXT** softkeys to move to the next or previous character space. Press **ENTER** to save the new name and return to the main menu.

Erasing an Existing Directory

Programs or complete directories may be erased to vacate program space for future use.

1. Select **PROG** from the Main Menu and then use the **RIGHT** arrow keys to move the cursor to **COPY** from the program menu. Press **ENTER** to select.

2. Use the **LEFT** and **RIGHT** softkeys to move the cursor to **ERASE DIR** and press **ENTER** to select.

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3. Select the relevant directory by moving the cursor with the **UP** and **DOWN** softkeys and press **ENTER.**

4. A final confirmation screen allows you either **KEEP** or **ERASE** the directory.

5.3 Programs

The programs in the Px2 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 up to 99 cycles and a 'hold' added to the end of the stage.

The Px2 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.

Advanced editing features (adjusting ramp speeds, setting a gradient of annealing temperatures, changing time/temperature with successive cycles) can be accessed by pressing the **ADVANCED** softkey at the relevant step (see section 5.4 for further details).

Creating a New Program

1. From the Main Menu select **PROG** using the appropriate softkey.
2. Move cursor to **EDIT**, then press **ENTER** to select.
3. Move the cursor to **NEW**, then press **ENTER** to select.
4. Move the cursor to **NEW PROG**, then press **ENTER** to select.
5. Move the cursor to desired target directory and press **ENTER** to select.
6. The instrument allocates a program number of the next available program space. You are now able to enter the user or protocol name, using the alphanumeric keypad. Use the **PREVIOUS** and **NEXT** softkeys to move to the position of the cursor where the character is to be added. Press **ENTER** only when the name is complete. If no characters are entered, the default NO NAME will be entered. The **BACK** button may be used to return to the previous screen if necessary.

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Note: If there are no free program spaces the Px2 will prompt you to overwrite an existing program.

7. Enter temperature (e.g. 95°C) and time (hr:min:sec), as required. Use the **LEFT** softkey to move the cursor as desired or **ENTER** to advance to the next step. The **UP** softkey can be used to scroll backward to correct a mistake. The program will not allow you to advance to step 2 until a valid temperature and time have been entered.

   Note: *Pressing the* ***ADVANCED*** *softkey at any step throughout the programming provides access to the advanced edit features. These facilitate the creation of more complex protocols including touchdown or gradient methods (see section 5.4 for more details).*

8. Continue to enter the desired time and temperature STEPS of this STAGE. If no more STEPS are required in the STAGE press **ENTER** (leaving each parameter at 0:0) until you access the number of cycles screen.
9. Enter the number of times you would like to cycle through the STAGE using the alphanumeric keys. A hold step can also be programmed after each stage so once the cycles have been completed the Px2 will wait at the specified temperature until manually advanced. This is particularly useful for 'hot start' protocols. If a hold step is not required, press **ENTER** to reach the next stage.
10. Continue adding steps to the new stage as per stage 1.
11. When all the steps have been entered in the last stage entering values of 0:0 will again bring access to the number of cycles required screen. 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. When **ENTER** is pressed, a new STAGE and STEP is displayed. By ENTERING values of 0:0 in the first step of a new stage the SAVE screen is accessed.
12. Press **SAVE** to save the program or **DISCARD** to abort the program. Pressing MENU at any time aborts the programming.

Editing an Existing Program

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Existing programs can be altered or overwritten by using the **EDIT** function. Please note that changes made are permanent and the original program is not retained. If the original program is still required, the **COPY** function should first be used to save the program to a different program space (see Section 5.3.4) 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 amending the current entry.

Advanced editing features (adjusting ramp speeds, setting a gradient of annealing temperatures, changing time/temperature with successive cycles) can be accessed by pressing the **ADVANCED** softkey at the relevant step (see section 5.4).

It is possible to edit programs whilst the Px2 is running a different protocol.

1. To edit and overwrite existing programs select **PROG** from the Main Menu using the appropriate softkey.
2. Select **EDIT** from the Program Menu by pressing **ENTER**.
3. Select **EXISTING** from the Program Editing Menu by pressing **ENTER**.
4. Use the **UP** and **DOWN** keys to move the cursor to the appropriate source directory and press **ENTER** to select. The **BACK** button may be used to return to the previous screen if necessary.
5. The keypad numbers or **PREVIOUS** and **NEXT** softkeys can be used to select the number of the program to be edited. Press **ENTER** to select the program.
6. Use the alphanumeric keypad in combination with the **PREVIOUS** and **NEXT** softkeys to edit the program name. Press **ENTER** to proceed or **BACK** to return to the previous screen.
7. Use **ENTER** to advance through the fields and the LEFT softkey to move across the menu. Time and temperature parameters can be altered using the numerical keys as required. The **UP** softkey can be used to move the cursor back to previous screens.

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

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

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9. Enter any changed cycle number or hold temperature values. Press **ENTER** to proceed to the next stage.
10. Edit steps in further stages as required. Once changes are completed the **SAVE** button can be pressed to access the save screen.
11. Press the **SAVE** softkey to save changes or **IGNORE** to keep the original program. Pressing **MENU** at anytime aborts the editing.

Viewing an Existing Program

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

1. Select **PROG** from the Main Menu and then use the **RIGHT** arrow keys to move the cursor to **VIEW** from the program menu. Press **ENTER** to select.

2. Use the **UP** and **DOWN** softkeys to select the appropriate directory and then press **ENTER**.

3. Use the **PREVIOUS** and **NEXT** softkeys to select the program and press **ENTER.**

4. Use the **NEXT** softkey to scroll through the program information. Press **QUIT** or **MENU** at any time to return to the Main Menu.

Copying Programs

Copying programs is necessary in, for example, modifying the pre-set programs described in Appendix V.

1. Select **PROG** from the Main Menu and then use the **RIGHT** arrow keys to move the cursor to **COPY** from the program menu. Press **ENTER** to select.

2. Move the cursor using the **UP** and **DOWN** softkeys to select the *source directory* and press **ENTER**.

3. Choose the program to be copied by using the **PREVIOUS** and **NEXT** softkeys and press **ENTER** to select.

4. Move the cursor using the **UP** and **DOWN** softkeys to select the *target directory* and press **ENTER**.

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5. Scroll through the possible program locations using the **PREVIOUS** and **NEXT** softkeys and press **COPY**. All empty file locations are marked as empty. It is possible to overwrite full program locations. A confirmation screen checks whether you wish to overwrite the data; press **COPY** or **ABORT** as desired.

Erasing an Existing Program

Programs or complete directories may be erased to vacate program space for future use.

1. Select **PROG** from the Main Menu and then use the **RIGHT** arrow key to move the cursor to **ERASE** from the program menu. Press **ENTER** to select.

2. Again use the **RIGHT** arrow key to move the cursor to **ERASE PROG** and press **ENTER** to select.

3. Select the directory containing the relevant program by moving the cursor with the **UP** and **DOWN** softkeys and press **ENTER.**

4. Use the **PREVIOUS** and **NEXT** keys to choose the relevant program and press **ERASE.**

5. A final confirmation screen allows you either **KEEP** or **ERASE** the program.

5.4 Advanced Edit Features

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.
- Gradient steps for gradient compatible blocks.

Advanced Edit features are accessed by pressing the **ADVANCED** softkey 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.

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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

Stage 2: *Enter parameters to create:*

95°C - 30s

55°C - 30s *x 11 Cycles*

72°C - 30s + 5s/cycle

1. To enter the +5s/cycle, select **PROG** followed by **EDIT**.
2. In the **EDIT** mode (NEW or EXISTING) move Stage 2, Step 3 (relevant extension step) using the navigation keys.
3. Press **ADVANCED** to access the Advanced Edit screen.

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4. Using the **UP** and **DOWN** softkeys, move the cursor through the fields until you reach the desired parameter. Use the **TOGGLE** softkey to alternate between Increase (INC) and Decrease (DEC) 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.*

5. Use **ENTER** or **BACK** 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

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50°C - 30s *x 5 Cycles*

72°C - 30s

1. In the **EDIT** mode (NEW or EXISTING) move to the temperature step of interest (Stage 2, Step 2).
2. Press the **ADVANCED** softkey to access the Advanced Edit screen. Navigate through the screen until you reach the **TEMP** field using the softkeys. Use **TOGGLE** to alternate between Increase (**INC**) and Decrease (**DEC**) in set point temp/cycle. Enter the desired value by which the set point temp per cycle should change (°C) using the number keys (minimum change is 0.1°C per cycle).
3. Move back to the step screen using the **ENTER** or the **BACK**. **<ADV>** will now appear on screen.

Changing Ramp Rates

Ramp rates can be altered in the Advanced Edit screen. 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.

5.5 Gradient Feature

This feature is only available to those who have purchased a 0.2ml or 0.5ml gradient block for temperature gradient cycling.

The gradient feature is designed to allow the rapid optimisation of annealing parameter (between 30 - 70°C) and can be accessed through the Advanced Edit function (see section 5.4). The minimum temperature to be assessed is entered along with the gradient spread (maximum of 15°C). 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:15>.**

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Gradient Programming Worked Example

1. In the **EDIT** mode (NEW or EXISTING) press **ENTER** to move to the relevant annealing temperature step. The annealing temperature entered in this step should 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 **ADVANCED** to access the Advanced Edit screen.
2. Enter your required gradient range from 1-15°C under **GRAD.**
3. The Program Edit screen will now display the gradient spread entered 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 section 5.3.

Invalid Gradient Parameters

If the combination of the Set Annealing Temperature and Gradient results in the block operating outside the temperature limits of 30 to 70°C, the Px2 alters the temperatures to remain within these limits. E.g. SET TEMP = 60°C and GRAD = 15°C. The SET TEMP will be changed to 55°C.

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.

1. To access the Gradient Calculator select **OPTS** from the MAIN MENU.
2. Move the cursor to **CALC** and press **ENTER**.
3. The block type you are using will automatically be displayed. Enter the annealing temperature and gradient range used in your optimisation experiment.
4. The well temperatures are displayed in sequence in a bar chart formation from column 1 to 12 with the calculated temperature for each column displayed to the left of the bar.
5. Press **BACK** to return to the Gradient Calculator screen to enter new parameters or alternatively press **MENU** to return to the Main Menu screen.

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The Gradient Calculator may be accessed at any time, whether the block is active or inactive.

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CHAPTER 6
Px2 THERMAL CYCLER
Running a Program on the *Px2*

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 to be used with your protocol.
- 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. It is possible to edit programs whilst the Px2 is running a different protocol (see Section 5.3).

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 Px2 – Short Instructions

Temperature control and heated lid functions are referred to in more detail in section 6.4.

1. Select **RUN** from the MAIN MENU.
2. Use the **UP** and **Down** softkeys to select the source directory and press **ENTER**.
3. Use the PREVIOUS and NEXT softkeys to select the program and press ENTER or the DOWN softkey.
4. Select the heated lid operating method, whether the heated lid is on or off during the final hold step, loading and end run settings, using the PREVIOUS and NEXT softkeys to scroll through options and ENTER to confirm selection.

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5. Select the temperature control mode using the PREVIOUS and NEXT softkeys. Pressing ENTER starts the run. BACK returns you to the previous screen if necessary.

6.3 Run Screens

When a program is running two different run screens can be displayed. The first screen is the text screen, which contains all the 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 Px2 and to determine the time of the end of the program. The second screen is the graphical display which shows the block and tube / sim tube temperatures. The screens may be toggled between using the **LEFT** softkey (**TEXT / GRAPH**).

These screens can be accessed from the* Main Menu *by pressing the RUN INFO softkey.

Text Screen

The current block performance screen displays the following:

- Program number and name.
- Status of the program.
- The temperature control mode being used.
- Target (programmed) temperature for a given step.
- The block and tube temperatures.
- Time remaining at current setpoint.
- 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.
- Time now.
- The calculated run end time (an estimated value which is updated throughout the run).

During a gradient step the run screen also displays the following:

- Target temperature range of the set gradient.

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- Temperature range achieved.
- The actual sample temperature as defined by the tube thermistor in position A2 of the block (when Active Tube Control has been selected for a run).

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.

End of Run Screen

The Px2 displays a further screen at the end of the run, which provides the following:

- The program name and number.
- The total run time.
- The maximum and minimum temperatures recorded during the run.
- Power interrupt information if a power failure occurred.

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 (ACTIVE 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.

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Note: <u>Please do not use aqueous solutions in the control tube thermistor</u>. We recommend that mineral oil be used in the tube containing the thermistor because the software is tuned to take into consideration the thermal conductivity of the mineral oil. In addition, PCR buffer can often cause corrosion of the thermistor, thus reducing the life expectancy of the probe.

The thermistor acts as a sample mimic, monitoring the sample temperatures as it changes during cycling, feeding back this information to the Px2 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 6.1*). When designing/transferring to a tube control program it is essential to understand the difference 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 Px2 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 Active 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 HBTC3372 tube for 0.2ml blocks or a HBTC3505 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 Px2 thermal cyclers.

Extension Lead for the Tube Thermistor

The Px2 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 7.3 for further details.

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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.

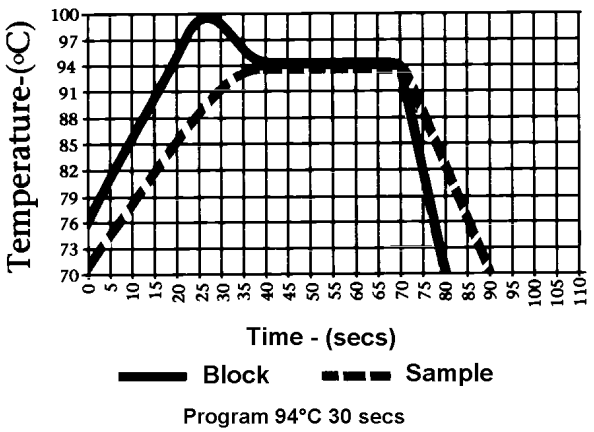
Reactions using Simulated Tube Control do not require the tube thermistor to be connected, but sample loading and volume details must be entered when prompted by the run screen. For all tube reactions in a 0.2ml or a 0.5ml thin walled tube, the volume factor is the **total reaction volume in** ml in one well, including any oil overlay.

Simulated Plate Control (SIM PLATE)

This mode of control operates on a similar principle to simulated tube control (*see Figure 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 with thin walled polypropylene plate.

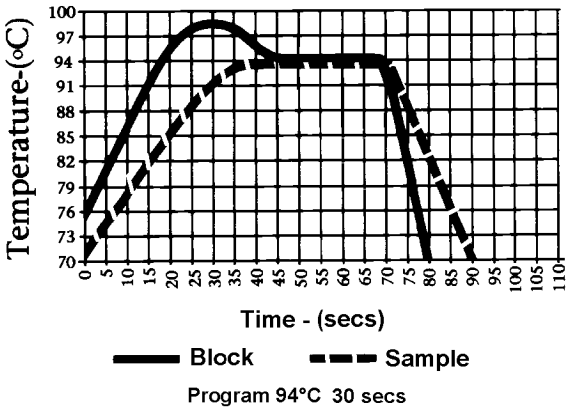
Figure 6.1 Modes of Control used with the Px2

a. Active Tube Control

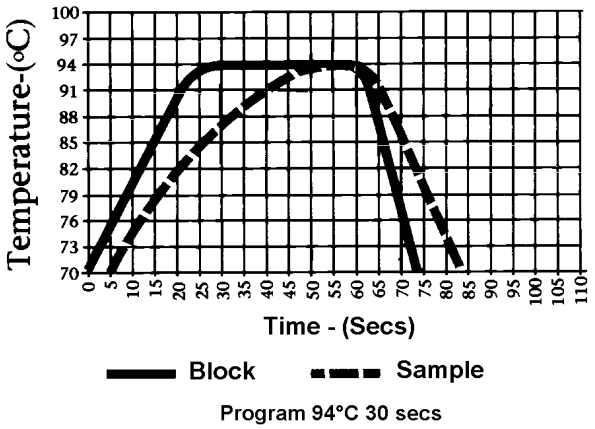


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b. Simulated Control (Tube, Plate, Slide)



c. Block Control



Simulated Slide Control (SIM SLIDE)

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 25 (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.

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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.

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 **START** 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 **CONTINUE** key has not been pressed to start a program, a manual start time out screen is displayed and the run is cleared.

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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 Px2 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°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°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°C incubation to ensure completion of the final extension step of a reaction.

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 **ABORT** aborts the program and returns you to the Main Menu screen. Pressing **CONTINUE** returns you to the Run screen and continues the run.

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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
Px2 THERMAL CYCLER
Troubleshooting

7.1 Programs Using ACTIVE Tube Control

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 as this can reduce the life expectancy of the thermistor.** 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 Px2 block accommodates most types of reaction tubes. The tube thermistor supplied with the Px2 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 Px2 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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7.2 Programs Using SIM Temperature Control

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.

Simulated Plate

0.2ml blocks

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

0.5ml blocks

For 0.2ml 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.

Simulated Slide

For 0.8mm - 1mm thick glass slides using simulated slide control the calibration factor is 25 (default value), other thicknesses will need optimisation.

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 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.

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7.3 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 Px2 as a temperature probe in the block control machine as follows:

1. Set the Px2 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 Px2.

3. Place the thermistor tube probe into the corresponding thermistor probe socket of the Px2, 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 Px2 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 Px2. Doing this will significantly reduce the total time required to run a protocol.

7.4 Optimisation of Protocols

The capability of the Px2 programming enables protocol optimisations to be performed very rapidly. Typically, temperature cycling protocols may consist of three distinct stages:

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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. This is best performed using the gradient feature described is section 5.5.

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
Px2 THERMAL CYCLER
Maintenance

8.1 General Cleaning

Before using any cleaning or decontamination method except those recommended below, please contact your local Thermo Hybaid office or authorised dealer to check that the proposed method will not damage the equipment.

1. All surfaces of the Px2 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 Px2 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 minimise 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 minimise 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 Laboratories Ltd, Conway

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Street, Hove, East Sussex BN3 3LY Tel: +44 (0) 1273 739 241, Fax: +44 (0) 1273 722 088) or **PCC-54** (Pierce Chemical Company). 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.

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 Px2 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 Px2 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

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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:

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
Px2 THERMAL CYCLER
Technical Specifications & Ordering Information

Description	**Px2 with 0.5ml Block**	**Px2 with 0.2ml Block**
Catalogue Number	HBPX2	HBPX2
Block Capacity	48 x 0.5ml tubes	96 x 0.2ml tubes
	96 x 0.3ml tubes	1 OmniUltra Plate
	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	0.1°C	0.1°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
Ingress Protection Rating	20	20
Standard Accessories	Tube Thermistor 0.5ml	Tube Thermistor 0.2ml
	(HBPXTTM05)	(HBPXTTM02)
Interchangeable Block Modules	HBPXB05	HBPXB02
Px2 Chassis	HBPX2	HBPX2

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Description	**Px2 with In Situ Block**	**Px2 with 384 Well Block**
Catalogue Number	HBPX2	HBPX2
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	0.1°C	0.1°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
Ingress Protection Rating	20	20
Standard Accessories	Humidity Chamber	
Interchangeable Block Modules	HBPXBFB	HBPXB384

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Description	**Px2 with 0.5ml Gradient Block**	**Px2 with 0.2ml Gradient Block**
Catalogue Number	HBPX2	HBPX2
Block Capacity	48 x 0.5ml tubes	96 x 0.2ml tubes
	96 x 0.3ml tubes	1 OmniUltra Plate
	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	0.1°C	0.1°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
Ingress Protection Rating	20	20
Standard Accessories	Tube Thermistor 0.5ml	Tube Thermistor 0.2ml
	(HBPXTTM05)	(HBPXTTM02)
Interchangeable Block Modules	HBPXBG05	HBPXBG02
Px2 Chassis	HBPX2	HBPX2

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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 and RS485
Alphanumeric programming	Yes
Gradient software	Yes
Power	550W
Dimensions (WxDxH)	240mm x 390mm x 280mm
Weight	8.6kg

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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 Px2 thermal cycler is removed from a cold room, it should be left to equilibrate for 2-3 hours to avoid condensation.

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APPENDIX I
Px2 THERMAL CYCLER
Recommended Consumables

Consumables	Pack Size	Description	Catalogue Number
Sealing Systems			
OptiTape with Dispenser	100	Sealing tape for OmniUltra Plate or other polycarbonate plates. Includes universal dispenser.	HB-TCT-100
	250		HB-TCT-250
OptiTape Refill	100	Refill roll of OptiTape sealing sheets for OmniUltra or other polycarbonate plates	HB-TCT-100R
	250		HB-TCT-250R
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

Plasticware

Consumables	Pack Size	Description	Catalogue Number
Plates			
OmniUltra - Non Irradiated	25	Polycarbonate 96 well plate – robot compatible	HB-TC-25D
OmniUltra - Gamma Irradiated	25	Polycarbonate 96 well plate – robot compatible	HB-TC-25N
OmniUltra Lid	25	Polycarbonate lids	HB-TC-25-NL
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

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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 OmniUltra Plate

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APPENDIX II
Px2 THERMAL CYCLER
Sealing Systems: The OmniSeal TD Mat & OptiTape

Overview

Thermo Hybaid offers several sealing systems to suit individual preferences. OptiTape is an optically clear single use adhesive tape suitable for use with 96 well polycarbonate plates, whilst the OmniSeal TD Mat is a reusable silicone rubber mat also for use with 96 well plates. For polypropylene plates we supply adhesive TDX Tape.

How to use the OptiTape and TDX Tape

OptiTape and **TDX Tape** are unique, single use sealing systems, designed for oil-free thermal cycling in 96 well plates and to prevent cross contamination between wells. After a thermal cycling reaction OptiTape or TDX Tape can be removed from the plate with ease.

OptiTape

Supplied on a roll (with optional dispenser) and designed for use with Thermo Hybaid's OmniUltra Plate, although will give an exceptional seal with other brands of **polycarbonate** plate.

TDX Tape

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

OptiTape 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 OptiTape and TDX Tapes must be used in combination with the reusable TD Compression Pad.

Sealing a 96 Well Plate

1. The tape can be applied to 96 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.

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2. The tape should be firmly anchored by pressing over each well. This can be done using a soft roller or by finger pressure. Especially with the OptiTape, ensure that no air pockets can be seen between the tape and the plate.

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

Place the sealed 96 well plate on the machine. Close the lid and select the 'Plates' icon. (Note: if an OmniTube 96 Plate is being used, the 'tubes' icon should be selected as the heated lid setting).

Removing the Tape

Prior to removing the Tape or OmniSeal Mat 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 Tape from the 96 well plate.

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.

Resealing Using OptiTape

OptiTape is designed for single use. Once heated and compressed, the backing conforms to the plate and cannot be easily repositioned. Reuse is therefore not advised.

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OmniSeal TD Mat

Cleaning & Sterilisation Procedures

The OmniSeal TD Mat (Catalogue No: HB-TD-MT-SRS-5) is used to seal 96 well plates when using the Px2 heated lid. Mats can be cleaned and sterilised using the procedures described below.

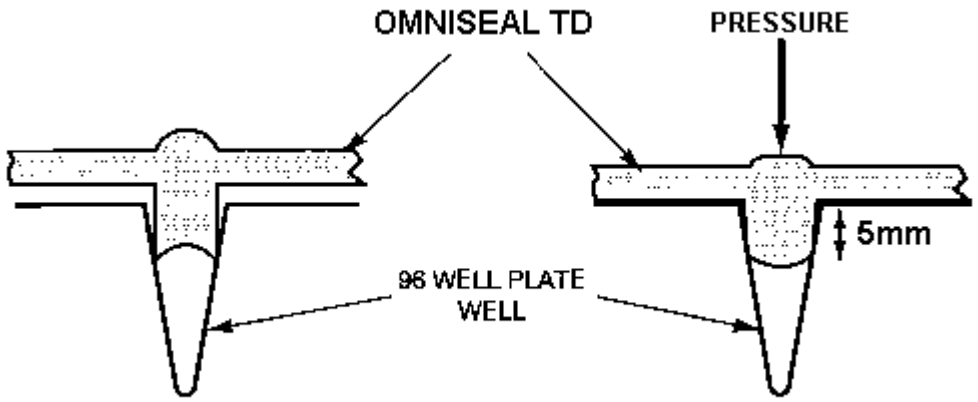
Soak for 1 minute in either 10% hypochlorite solution, or 0.1M hydrochloric acid solution and then rinse with distilled water. Dry inside an oven at up to 80^{o}C if required. Autoclave at 121^{o}C for 15 minutes.

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 Px2 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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Reusability of the OmniSeal TD Mats

The OmniSeal TD Mat can be reused 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 reused 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.

The Thermo Hybaid OmniUltra helps to address this problem to some extent. In our tests reaction volumes of 10µl gave strong results. 5µl reaction volumes gave visible but faint results thus 10µl is the minimum recommended sample volume for this plate.

This is because this plate is injection moulded which results in absolute well to well and plate to plate consistency. The well wall thickness of conventional vacuum moulded plates is inconsistent resulting in highly porous patches which allow large amounts of sample to pass through.

If you are working with sample volumes of less than 10µl and are using the OmniUltra, it may be advisable to switch to polypropylene consumables instead. For polycarbonate plates supplied by other manufacturers a minimum sample volume of 25µl should be observed to ensure adequate sample remains after PCR.

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APPENDIX III
Px2 THERMAL CYCLER
In Situ Block Application Notes

Overview

The Px2 *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 Px2 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 25. 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 Px2'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 Px2'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 Px2 *In Situ*, the denaturation temperature is reduced by 3°C.

Programming

Programming and operation of the *In Situ* module are identical to the standard Px2 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 (see section 4.7). For more complex multi-step incubations or for temperature cycling reactions a protocol can be designed and run using Simulated Slide Control (see 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
Px2 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 optimised 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 Px2 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.
OPTS	Allows the accessing of SETUP options or GRADIENT CALCULATOR Software

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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 Px2 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 Px2 (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.
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.

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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 Px2 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.
View	The View function allows the contents of programs to be accessed.

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APPENDIX V
Px2 THERMAL CYCLER
Pre-Set Programs on the Px2

Programs coded into the F:HYBAID Directory of the Px2 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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