Operation Manual

PCR Sprint Thermal Cycler

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PCR SPRINT TEMPERATURE CYCLING SYSTEM
PCR License Registration

This instrument, Serial No. ..... is an Authorized Thermal Cycler. Its purchase price includes the up-front fee component of a license under 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. Hoffman-La Roche Ltd, covering the Polymerase Chain Reaction (PCR) process, to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications' licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. 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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Applied Biosystems does not guarantee the performance of this instrument.

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Warranty

Thermo Electron Corporation guarantees that the PCR Sprint Temperature Cycling System you have received has been thoroughly tested and meets its published specification.

This warranty is valid for 12 months* only if this product and functions have been used according to the user instruction manual.

This warranty period can be extended to a total of 24 months (free of charge) by completing the warranty registration card supplied with the instrument, also available online at www.thermo.com/warrantylog.

No liability is accepted for loss or damage arising from the incorrect use of the PCR Sprint Thermal Cycler. Thermo's liability is limited to the repair or replacement of the unit or refund of the purchase price at Thermo's option. Thermo Electron is not liable for any consequential damages.

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

The Thermo Electron PCR Sprint is for research use only.

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

NB: The PCR Sprint should only be used by suitably qualified and trained people. If the PCR Sprint is not used as specified in this Manual, the protection provided by the equipment may be impaired (see Chapter 2).

* Excludes tube thermistor accessory, which carries a warranty for 90 days only.

Contents

PCR Sprint Thermal Cycler

1. System Description

1.1 Overview

PCR Sprint is designed for fast, accurate, licensed thermal cycling of small numbers of samples. PCR Sprint features high-speed sub-ambient blocks to perform oil-free thermal cycling with excellent dynamic uniformity and precision control of sample temperature.

1.2 PCR Sprint System

The PCR Sprint System consists of 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 two types of block modules, which have the following sample formats:

The 0.2ml Block Module: holds 24 x 0.2ml individual OmniTubes.

The 0.5ml Block Module: holds 20 x standard 0.5ml individual OmniTubes.

The temperature cycling blocks for the system is of specially coated precision-machined aluminium, ensuring the best possible fit of reaction tubes in the block. This enables rapid and accurate heat transfer from the block to the samples.

Accurate sample temperature control in 0.2ml and 0.5ml block types is achieved by proprietary **Active Tube Control** software. The tube thermistor probe monitors the temperature within a dummy sample tube and this information is fed back to precisely control the block temperature to achieve the optimum cycling profile. This feedback loop allows the transition to target temperature to be accelerated by creating a temperature gradient between the tube temperature and the block temperature.

Alternatively, **Simulated Tube Control** may be used for reactions when it is not appropriate to use the tube thermistor probe (for example in very small (<20µl) reactions). 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.

Heating and Cooling

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

The sub-ambient aluminium block is heated and cooled by the latest in Peltier technology. The block modules excel in performing applications such as RAPD and Differential Display, which require cycling temperatures close to ambient. It mirrors the temperature control characteristics of larger Thermo blocks in thermal cycling applications, allowing protocols to be directly transferred. 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 down to 4°C.

Programming and Operation

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

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

The unit has program space for up to 60 full cycling protocols including the preset protocols. Programs can be saved in one of five user directories, each of which can be identified by user or protocol type.

Preset Programs

The PCR Sprint is supplied with 10 non-editable preset protocols stored as programs 51-60 in the F:THERMO directory. These protocols cover the most common thermal cycling techniques and can be used to create customized protocols by editing (see section 5). Full details of the cycling parameters of each can be found in Appendix III.

Advanced Edit - Temperature Ramping

The temperature ramping (Ramp Rate) enables the rate of change of sample temperature (degrees centigrade per second) to be slowed down.

Under normal circumstances the temperature cycling times are very rapid, which minimizes non-specific reactions. The rate of sample temperature change during thermal cycling is controlled so that it is as fast as possible without affecting the block uniformity and accuracy. In some instances, however, it may be advantageous to limit the rate of change of temperature, for example, to allow limited extension of short or degenerate primers between primer annealing and DNA synthesis steps to stabilize 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. For example, 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 to perform TouchDown PCR in which it is required to decrease the annealing temperature over successive cycles.

Temperature Control

Temperature accuracy and uniformity across the block, particularly during rapid temperature cycling operations, have been of paramount importance during the design and development of the PCR Sprint. Every machine is calibrated using a number of thermistor probes, located in tubes, which are placed at several block positions to ensure that the required temperatures and incubation times achieved are identical in all samples.

1.3 The Heated Lid

The unit can be operated with or without the lid being activated. The heated lid enables PCR Sprint users to run temperature cycling protocols without the need for paraffin or mineral oil overlays. In a unit without a heated lid such vapour barriers are required to prevent evaporation of reaction constituents before thermal cycling is complete.

The heated lid operates by positioning a heated plate in intimate contact with the top of the reaction tubes or wells. The heating plate then raises the air temperature at the top of each tube to a temperature that is permanently higher than the sample temperature. The elevated air temperature, relative to the sample temperature, minimizes evaporation as the reaction mixture is repeatedly heated and cooled.

The lid is opened by pressing the black catch. On closing, the height of the heating plate inside the heated lid is automatically adjusted on light pressure springs. The heated lid is designed to be compatible with both reaction sample formats, i.e. 0.5ml tubes and 0.2ml tubes. In most cases, an experimental protocol is largely unchanged when switching from using oil overlay to an oil-free system.

⚠WARNING

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.

The temperature of the heating plate is set at 115°C (max. surface temperature).

The heating 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 3 minutes before cycling commences.

1.4 Safety

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

I Indicates the ON position of the main power switch.

O Indicates the OFF position of the main power switch.

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

⚠WARNING

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

ATTENTION: *Surface chaude.*

⚠WARNING

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

AVERTISSEMENT: *Risqué de choc electrique.*

1.5 Support Services

The PCR Sprint has been designed for reliability and for ease of maintenance. Thermo Electron Corporation continues to offer full service and technical support for all its products. International Sales, Services and Technical Support contact your local Thermo Electron Corporation subsidiary or authorized distributor. Visit www.thermo.com/molecularbiology for contact details.

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2. Installation

2.1 Unpacking

When the PCR Sprint is delivered you should first check that you have received everything undamaged.

⚠CAUTION
SAFETY NOTE: Do not attempt to operate the unit if it appears to have been damaged during transit.

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

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

2.2 Packing List

1. PCR Sprint Control Chassis
2. PCR Sprint Block Module (pre-installed into unit)
3. User Instruction Manual
4. Mains Lead
5. Control Thermistor Probe
6. Tube of Mineral Oil
7. Tube Selection Pack

If any item is missing or damaged, contact your local Thermo Electron Corporation office/authorized distributor.

Ensure any padding between the plate inside the heated lid and the block is removed before using the instrument.

2.3 Installation

Siting the Unit

The user interface for all programming and operating functions is the **Control Chassis**. The Control Chassis should be sited close to a suitable main supply. Where possible, avoid connecting the Control Chassis to a main supply subject to main voltage fluctuations, for example, a socket shared by an ultracentrifuge, refrigerator or -70°C freezer.

⚠WARNING

SAFETY NOTE: Before using the PCR Sprint for the first time check that the voltage rating of the instrument is correct for your supply (details are on the serial number label).

SAFETY NOTE: The unit should not be positioned in such a way as to restrict access to the power inlet.

- Excess heat is removed from the PCR Sprint by a fan. Allow at least 15cm of clearance between the sides of the unit and any object or wall, which may restrict the flow of air. Air is drawn into the unit underneath and expelled out of the sides. 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.
- All PCR Sprint 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 be closed during temperature cycling, even if not switched on.
- The PCR Sprint is intended for indoor use at an ambient temperature of 4-35°C in conditions of up to 80% humidity. These specifications have been calculated for operations at between 0 and 2000m altitudes.
- The PCR Sprint 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.

⚠CAUTION

SAFETY NOTE: If equilibration is not done, there is a risk that condensation may form within the unit and cause a short circuit.

PAT (Portable Appliance Test) Recommendations

This unit contains sensitive electronic components. The PCR Sprint should not be exposed to high voltage discharges, or serious damage will result. All PCR Sprint units are PAT tested prior to shipment. For more information contact Thermo Electron Corporation.

Powering the Unit

The PCR Sprint is a class 1 (Earthed) appliance. It must be connected to a protected earth connection via the supplied main cord.

Switch on the PCR Sprint at the power switch on the side of the unit. When first switched on the unit will complete a start up and self test routine.

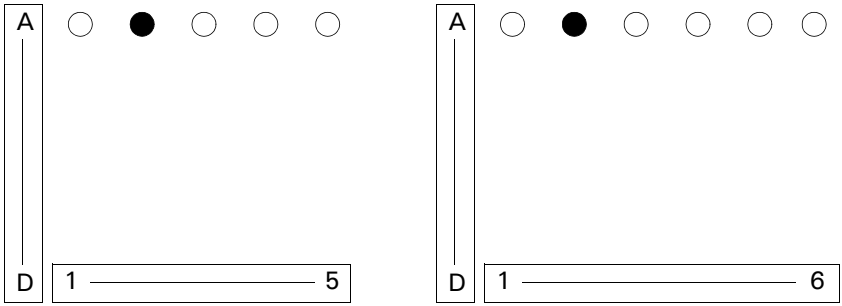
⚠WARNING

The block will become hot! Keep the lid closed during the self-test procedure.

The self-test routine checks the block thermistor, heating and cooling circuits and the Heated Lid. This takes approximately 1 minute.

Thermistor Positioning

The control thermistor probe (when required) should be inserted into the socket to the left of the block towards the rear of the instrument. Insert the tube with the thermistor probe into the block in position 'A2' (see fig 2.2). Ensure that the tube contains the same volume of mineral oil as the intended reaction volume, and that the probe is positioned in the center of the tube and immersed in the oil.



0.5 ml Block **0.2 ml Block**

Figure 2.2 Well Numbering Showing Tube Thermistor Positioning in A2

Block Interchange

⚠WARNING

SAFETY NOTE: Blocks should only be interchanged when the power to the instrument is switched off.

⚠WARNING

Do not tamper with the connector in the block recess when the block is removed as static discharge could damage sensitive electronic components inside the machine.

⚠WARNING

Block Heat Sink may be hot after use, even if the block itself is cold.

To remove the PCR Sprint interchangeable block:

1. Lift the lid and remove the two retaining thumbscrews on the upper surface of the block and the single retaining thumbscrew on the rear of the instrument.
2. Lift the block out of the chassis.
3. Disconnect the connection cable from the block.

Refitting a block is essentially the reverse procedure, but note that if the block type is changed (0.2ml to 0.5ml or 0.5ml to 0.2ml) then the calibration software must also be changed in the set-up menu once the block is installed.

3. Operation

3.1 Initial Start Up

At power on, the initialization screen identifying the unit will be displayed.

After 10 seconds a screen will display "PCR Sprint going through a self test procedure". For further details refer to Section 2.3 of this manual.

3.2 Main Menu

After successful start up, the first menu to be displayed is the 'Main' Menu. All aspects of the programming are accessed through the Main Menu. It is possible to return to this menu at any time by pressing 'MENU'. The Main Menu displays the status of the block (active, inactive, interrupted or active resumed) and allows access to the RUN, MAN, PROG and SETUP menus.

Table 3.1 Summary of Main Menu Functions

RUN	Select RUN to run an existing program.
MAN	Select MAN to run a manual incubation.
PROG	Select PROG to edit, copy, view or erase a program.
SET UP	Select SET UP to alter the power resume function, block type fitted and to identify software version fitted.

3.3 Operating Keys

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

3.4 Set Up Functions

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

Block Selection

The 0.2ml and 0.5ml blocks have different calibration settings, which must be selected whenever the blocks are interchanged.

1. Select BLOCK from the SETUP menu and press ENTER.
2. The 0.2ml or 0.5ml options can be toggled using the → key.
3. Press ENTER to save new settings and return to the Main Menu. Pressing the MENU key will return to the Main Menu without saving the changes.

Power Failure Resume

If there is a power interruption during a run, the PCR Sprint can continue a PCR protocol automatically from the point where the power was interrupted. The unit can be set to resume a program (at the cycle and set point where the interruption occurred) or to abandon the program.

1. Select POWER from the SETUP menu and press ENTER.
2. The ABANDON or RESUME options can be toggled using the → key.
3. Press ENTER to return to the Main Menu.

The instrument will record the step, stage and cycle number at which the power was interrupted. (See Section 4.4 for more information.) Note that if there have been several breaks in the power supply, only the most recent interruption is recorded.

3.5 Running a Program

At the Main Menu, with the cursor on RUN, press ENTER. Select the source directory (e.g. A:MYPROGRAMS) and press ENTER then select a program number to be run. You can scroll through the list of programs held in a directory using the arrow keys. At any stage prior to starting the program, pressing the MENU key will return to the Main Menu without starting the program. This topic is covered in more depth in section 4.

3.6 Manual Operation

This option is used for static incubations when thermal cycling is not required, e.g. probe denaturations, enzyme reactions. The unit will only operate under block control (see section 4 for details) in this mode, with the count-up timer starting when the block reaches temperature.

1. Select MAN from the Main Menu and press ENTER.
2. Enter the name of the user or protocol if desired using the ↑ and ↓ arrows to scroll through the characters.
   Move to the next/previous character using the ← and → arrow keys. (A maximum of 7 characters can be entered.) Press ENTER only when the name is complete. If no characters are entered, the default NO NAME will be entered.

3. Enter the temperature using the numeric keys. Press ENTER to continue.
4. Select the heated lid on (auto) or off using the ➔ key.
5. Press ENTER to start the incubation. The screen displays the set and actual temperatures of the block together with the elapsed time.

   NOTE:If the machine is started from 'cold', approximately three minutes will be required before the heated lid will be at operating temperature (based on 20°C ambient and 230V supply. This may vary under different power conditions). Condensation may be experienced if samples are loaded before the lid is at operating temperature.
6. Press STOP twice to cancel the manual program.
7. Press CONTINUE to change the set temperature.

See Figure 3.1 below.

FIGURE 3.1: Using the PCR Sprint for Static Temperature Incubations (Manual Control)

1. Press ENTER to select the MANUAL mode.

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t	
I	N	A	C	T	I	V	E												
						M	A	I	N		M	E	N	U					
R	U	N			M	A	N			P	R	O	G		S	E	T	U	P

2. Use ↑,↓,➔, and ← to enter name then press ENTER.

M	A	N	U	A	L		U	S	E	R			T	H	E	R	M	O	
T	E	M	P		3	7	.	0											
H	O	T	L	I	D		O	F	F										
<	E	N	T	E	R	>		T	O		C	O	N	T	I	N	U	E	

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

M	A	N	U	A	L		U	S	E	R			T	H	E	R	M	O	
T	E	M	P		3	5	.	0											
H	O	T	L	I	D		O	F	F										
<	E	N	T	E	R	>		T	O		C	O	N	T	I	N	U	E	

4. Use ➔ to toggle heated lid ON (auto) or OFF, then press ENTER.

M	A	N	U	A	L		U	S	E	R			T	H	E	R	M	O	
T	E	M	P		3	5	.	0											
H	O	T	L	I	D		O	F	F										
<	E	N	T	E	R	>		T	O		C	O	N	T	I	N	U	E	

5. Press CONTINUE to change the setpoint temperature, or STOP to stop the incubation.

M	A	N	U	A	L		U	S	E	R			T	H	E	R	M	O	
T	E	M	P		4	5	.	0		T	I	M	E		0	5	:	0	0
B	L	O	C	K		4	5	.	0										
<	C	O	N	T	I	N	U	E	>		N	E	W		T	E	M	P	

3.7 Programming Function

Temperature cycling programs of varying levels of complexity can be easily created using PCR Sprint. As with all operations of the PCR Sprint, programming is accessed from the Main Menu. Press MENU for the Main Menu screen to appear on the display.

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

- EDIT: To alter the details of an EXISTING previously stored program or to create a completely NEW program from scratch.
  Note that if NEW is used, it will automatically overwrite the previously stored program number entered. When in doubt, use the VIEW mode (see below) to check the program before overwriting.
- COPY: Use the screen prompts to copy between programs stored on the PCR Sprint.
- VIEW: To check the details of a previously stored program without risking accidental alteration of data.
- ERASE: To erase the details of a program, setting the parameters to zero.
  These are discussed in detail in section 5.

3.8 Loading Samples and Heated Lid Operation

The PCR Sprint is able to produce identical cycling profiles whether 1 or 24 samples are present on the block, and with or without oil-free operation.

Loading Samples

The following guidelines maximize the uniformity and thermal transfer characteristics of the block for different consumable types:

Load samples uniformly across the block rather than in clusters. Spreading the thermal load in this way maintains uniformity.

If small numbers of samples are to be run, use dummy tubes to ensure that there is at least one tube in each quadrant of the block. This ensures even heated lid pressure on the tubes.

Care should be taken in matching block type, consumable and control mode.

Ensure caps of tubes are properly closed before loading onto the block.

Heated Lid Operation

The heated lid is simple to operate. By closing the lid the heater plate is raised to its correct height in one action.

All that is required from a user perspective is to ensure that reaction tubes are seated correctly in the block, correctly capped. No adjustment is required for different height consumables.

4. Running a Program

4.1 The RUN Option

Reaction tubes should be distributed evenly in the block to obtain the most uniform results.

To run a stored program:

1. Select the RUN option on the Main Menu.
2. Use the arrow keys to select the source directory, the program number, and the heated lid setting and alarm options. (Also see Figure 4.1.)
3. Select the control method desired and enter the number of tubes and reaction volume if Simulated Tube Control is to be used (see Table 4.1). Once a program is selected, the program name will be displayed and lid preheating (if selected) will commence.

FIGURE 4.1: Running a Program on the PCR Sprint

1. Press ENTER to select the RUN mode.

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t	
I	N	A	C	T	I	V	E												
						M	A	I	N		M	E	N	U					
R	U	N			M	A	N			P	R	O	G		S	E	T	U	P

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

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

3. Enter the program number using the number or ↑,↓ keys. The program name will appear (if assigned), then press ENTER.

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

4. Select the heated lid operating method, loading and end run alarm settings using → to toggle the options, then press ENTER to select.

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

5. Use ➔ to select the control mode, then press ENTER.

R	U	N					A	:	0	2		T	H	E	R	M	O
						T	U	B	E								
P	R	E	S	S		➔		T	O		C	H	A	N	G	E	
<	E	N	T	E	R	>		T	O		C	O	N	T	I	N	U	E

IF a simulated method is selected, the number of samples and the sample volume needs to be entered using the number keys. Press ENTER to proceed.

R	U	N					A	:	0	2		T	H	E	R	M	O
					S	I	M		T	U	B	E					
N	O		O	F		S	A	M	P	L	E	S		9	6		
S	A	M	P	L	E		V	O	L	U	M	E		0	5	0	

6. Cycling will commmence if the heated lid is selected OFF. IF selected ON, the heated lid will then preheat.
   - If MANUAL start is selected, ENTER will need to be pressed before cycling will begin.

   - If AUTO is selected, the process will being automatically.

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

R	U	N	N	I	N	G		A	:	0	2		T	H	E	R	M	O
T	U	B	E							T	E	M	P			9	5	.	0
T	I	M	E						B	L	O	C	K			8	2	.	4
0	:	0	0	:	3	0			T	U	B	E				6	8	.	2

4.2 Temperature Control Options

An understanding of the temperature control methodology is crucial to the accurate operation of a thermal cycler. A choice of methods is offered:

Active Tube Control (TUBE)

Recommended for all reactions above 20µl volume in 0.5ml and 0.2ml tubes. This type of control uses the remote thermistor probe mounted in an appropriate tube. A volume of mineral oil equivalent to the volume in the reaction tube must be present in the control tube

(Do not use aqueous solutions with the control tube thermistor.)

The thermistor acts as a simple mimic, monitoring the sample temperature as it changes during cycling, feeding back this information to the PCR Sprint processor. This feedback allows the unit to respond to the sample temperature and hence ensure that the samples achieve the exact temperatures and times programmed. To bring the sample to temperature rapidly, the block is heated/cooled to beyond the set temperature for the sample. This can be observed on the display of the PCR Sprint during cycling.

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 PCR Sprint or a thermal cycler without tube control, there will be a lag between the block reaching target temperature and the sample reaching target temperature. Thus when transferring protocols from a block control machine the incubation times may be reduced by up to 50%, and in some cases the temperatures adjusted slightly.

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

The reaction tube containing the thermistor probe should be changed periodically e.g. every 5-10 cycling reactions, as with prolonged use its fit in the block may deteriorate, particularly if using thin-walled tubes. The reaction tube used for the thermistor probe should be of the same type as used for the sample reaction tubes.

When you receive your unit, the thermistor is mounted in a HB-TC-3372 tube for 0.2ml blocks or a HB-TC-3505 tube for 0.5ml blocks. We recommend changing your complete tube thermistor assembly after one year's use (Tube thermistor 0.5ml HB-PX-TTM05/Tube thermistor 0.2ml HB-PX-TTM02).

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

NOTE:Tube thermistors from TouchDown, OmniGene and Omn-E instruments are not compatible and can NOT be used with PCR Sprint thermal cyclers.

Extension Lead for the Tube Thermistor

The PCR Sprint has an optional extension lead for the thermistor. This may be used for transferring protocols from instruments that cannot use Active Tube Control. See Section 6.1 for further details.

Simulated Tube Control (SIM TUBE)

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

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

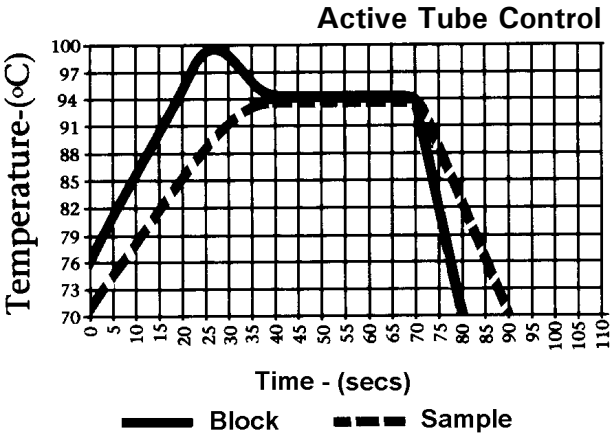
Reactions using Simulated Tube Control do not require the tube thermistor to be connected, but sample loading and volume details must be entered when prompted by the run screen. For all tube reactions in a 0.2ml or a 0.5ml thin walled tube, the volume factor is the total reaction volume in µl in one well, including any oil overlay. (*See Table 4.1*) For reactions in thick walled 0.5ml tubes, use (volume + 50) µl as a good approximation to achieving the same profile.

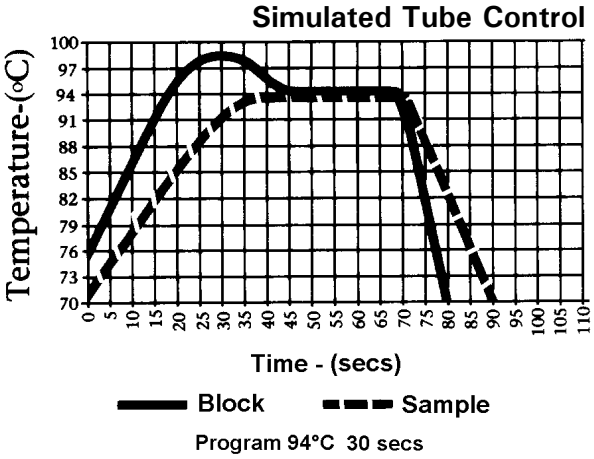
Table 4.1: Guidelines for Selecting Temperature Control Method and Entering Volume Factors

		Control Method	Loading Factor	Volume Factor
Consumable Type			0.5ml Block	0.2ml Block
0.5ml thin walled	SIM TUBE	No. of tubes 1-20	vol. (µl)	-
0.5ml thick walled	SIM TUBE	No. of tubes 1-20	vol. (µl) + 50	-
0.2ml thin walled	SIM TUBE	No. of tubes 1-24	-	vol. (µl)

⚠WARNING

Care should be taken when using 35S labelled primers as they can form volatile breakdown products. These diffuse through the walls of the reaction vessel (polycarbonate or polypropylene) and bind chemically to the block. (See section 7.)





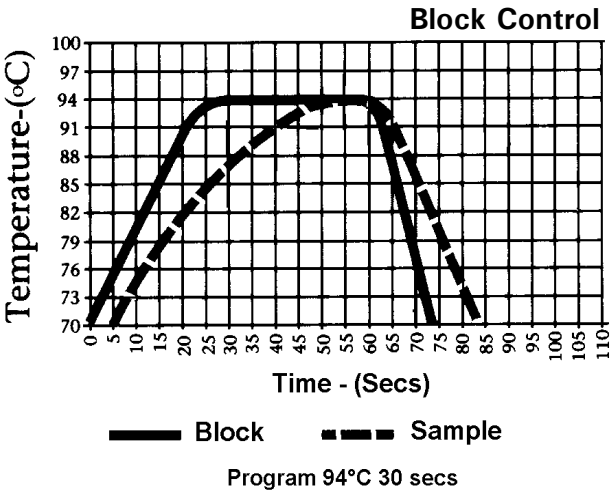


Figure 4.2 Modes of Control used with the PCR Sprint

Block Control

Simply controls block temperature as on a conventional dry block machine. We would not recommend this means of control for thermal cycling because of the variability in thermal profile obtained with different sample volumes and consumable types.

4.3 Heated Lid Preheat

Once a program and block have been selected, (and sample number and volume entered where appropriate) the program will proceed according to the set-up conditions as outlined below.
The heated lid typically takes approximately 3 minutes to reach operating temperature. During this time, the block is cooled to 4°C.

⚠WARNING

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.

Heated Lid - Automatic Start

This selection is the choice for robust cycling reactions, where the reactants can withstand being incubated for the duration of the preheat (about 3 minutes) without the generation of spurious products. After the program is selected, the heated lid will preheat. When the lid reaches operating temperature, 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 selection is the choice for sensitive cycling reactions, where the reactants will not tolerate being incubated at low temperatures even for a short time, or where the risk of non-specific reactions must be eliminated. After the program is selected, the heated lid will preheat. The screen will indicate when the lid has finished its heating cycle. The ENTER key will need to be pushed to start the PCR protocol. In manual start mode the samples should be mixed, loaded and the lid closed after the preheat is completed.

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

No Heated Lid

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

4.4 Run Screens

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

A fourth screen becomes available if the run has been interrupted by a power failure (see Section 3.4).

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

FIGURE 4.3: Main Run Screens Displayed by the PCR Sprint

(1) Current Block Activity

R	U	N	N	I	N	G		A	:	0	1		T	H	E	R	M	O	
T	U	B	E							T	E	M	P			9	5	.	0
T	I	M	E						B	L	O	C	K			9	5	.	3
0	:	0	0	:	1	4			T	U	B	E				9	4	.	9

(2) Program Progress and Performance

S	T	A	G	E		0	2						S	T	E	P		0	2
C	Y	C	L	E	S		C	O	M	P	L	E	T	E	D			2	1
C	Y	C	L	E	S		R	E	M	A	I	N	I	N	G			0	9
M	A	X		9	4	.	9					M	I	N		5	4	.	2

(3) Estimated Run Time Information

R	U	N	N	I	N	G		A	:	0	1		T	H	E	R	M	O	
P	R	O	G	R	A	M		E	N	D		I	N		0	1	:	2	7

Screen 1

The current block activity displays the following information: -

Program number and name.

Control method i.e., TUBE, SIM TUBE or BLOCK.

Current block and tube temperatures.

Set point (programmed) temperature.

Time remaining at current set point.

Screen 2

The program progress and performance screen displays the following information: -

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.

Screen 3

The estimated run time screen displays the following information: -

The calculated time until run end (an estimated value, which is updated throughout the 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. (See Section 3.3 for more information.) It contains the following information: -

The stage, steps and cycle in the run when the power failure occurred.

4.5 End of Run Screen

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

The program name and number.

The total run time.

The maximum and minimum temperatures recorded during the run.

Block Idle Screen

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

Error Screens

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

4.6 Hold & Pause Function

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

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.

4.7 Aborting Programs

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

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

4.8 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.

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.

5. Programming

5.1 Introduction

The programming interface on the PCR Sprint has been designed to be easy to use. The memory will hold up to 60 complete thermal cycling protocols including 10 pre-programmed template programs (Nos. 51-60 detailed in Appendix III). Programs are identified on the screen by a number (01-60) and a user defined name (up to 7 characters). Each program is assigned to a directory to group programs by user name or protocol type.

To run a program you may select an existing program, edit an existing program or create a new program. Additionally the PCR Sprint can be run in 'Manual' mode at a single temperature for static incubations (See Section 3.6).

The PCR Sprint interface also includes a number of advanced program features detailed in Section 5.9.

5.2 Steps & Stages of a Program

The programs in the PCR Sprint 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 associated with a typical protocol i.e. 95°C, 55°C and 72°C for 30 seconds each. In addition each stage can be repeated for up to 99 cycles, and a 'hold' added to the end of the stage.

The PCR Sprint allows you to program up to 5 separate stages each with up to 5 separate steps in each stage. This means that even the most complex thermal cycling protocol may be saved in a single program space.

5.3 Directories

The programs in PCR Sprint can be grouped by user name or protocol type in one of five directories A-E, with a sixth directory, F, reserved for preset programs. This facilitates efficient management of the program memory space.

Directories can be activated and named from within the EDIT and NEW menu, Use the cursor keys to assign the name in the same way as for naming programs (see 5.4 below). 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.

5.4 Creating a New Program

1. To create a new program select PROG from the Main Menu, select EDIT and NEW from the Program Menu, and enter the desired target directory.
2. The instrument allocates the number of the next free program space. You are now able to enter the name of the user or protocol, using the ↑ and ↓ arrows to scroll through the characters. Move to the next/previous character using the → and ← arrow keys. Press **ENTER** only when the name is complete. If no characters are entered, the default NO NAME will be entered.

   Note that if there are no free program spaces the PCR Sprint will prompt you to delete an existing program before recommencing editing.

3. Enter the temperature and time of the first step of your protocol.
   The arrow keys can be used to scroll backward or forward to correct a mistake.
   Note that the program will not allow you to advance to step 2 until a valid temperature and time have been entered.
4. Press **ENTER** to move to step 2 of the protocol.
5. Continue to enter the remaining times and temperatures of this stage. Up to five steps may be entered, although three steps are more typical.
6. Enter the number of cycles required.
7. Enter the hold temperature if required (see Section 4.5) or leave at 0.0. Press **ENTER**.
8. Repeat for each stage required. Up to five stages may be entered. One is common although three are needed if initial denaturation and final extension steps are required.
9. Press **ENTER** to save the program.

FIGURE 5.1: Creating Programs on the PCR Sprint using the "NEW" Program Option

1. Move the cursor to PROG, then press ENTER to select.

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t
						I	N	A	C	T	I	V	E					
						M	A	I	N		M	E	N	U				
R	U	N			M	A	N			P	R	O	G		S	E	T	U	P

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

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t
						I	N	A	C	T	I	V	E					
				P	R	O	G	R	A	M		M	E	N	U			
E	D	I	T		C	O	P	Y		V	I	E	W		E	R	A	S	E

3. Move the cursor then press ENTER to select NEW program to edit.

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t
						I	N	A	C	T	I	V	E					
P	R	O	G	R	A	M		E	D	I	T	I	N	G		M	E	N	U
E	X	I	S	T	I	N	G			N	E	W							

4. Use the ↑,↓ keys to select the source directory then press ENTER.

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

5. Use cursor keys to enter the program name. Press ENTER to proceed. If no data is entered, a default NO NAME will appear.

N	E	W					A	:	0	5							
S	T	A	G	E		0	1			S	T	E	P		0	1		
T	E	M	P		0	0	:	0										
T	I	M	E		0	:	0	0	:	0	0							

6. Enter temperature (e.g. 94.5), and time (hr:min:sec), as required. Use → to move across menu, or ↓ to advance to the next step.

N	E	W					A	:	0	5		P	C	R		6	8	C
S	T	A	G	E		0	1			S	T	E	P		0	1		
T	E	M	P		9	4	.	5										
T	I	M	E		0	:	0	1	:	0	0							

7. Continue adding steps as required for stage 1 (max. 5 steps per stage).

N	E	W						A	:	0	5		P	C	R		6	8	C
S	T	A	G	E		0	1				S	T	E	P		0	2		
T	E	M	P		6	8	:	0											
T	I	M	E		0	:	0	1	:	0	0								

8. Press ENTER (or ↓ key) with each parameter at zero to finish stage 1.

N	E	W						A	:	0	5		P	C	R		6	8	C
S	T	A	G	E		N	U	M	B	E	R						0	1	
N	U	M	B	E	R		O	F		C	Y	C	L	E	S		0	3	0
H	O	L	D		T	E	M	P			0	0	.	0					

9. Enter number of cycles of stage 1, and press ENTER.
Enter a hold temperature if required (4° C-99° C)
NOTE: During operation, hold temp is maintained until CONTINUE is pressed. Press ENTER to go to the next stage.

N	E	W						A	:	0	5		P	C	R		6	8	C
S	T	A	G	E		0	2				S	T	E	P		0	1		
T	E	M	P		7	2	:	0											
T	I	M	E		0	:	0	5	:	0	0								

10. Program steps for stage 2 (further stages as required to a maximum of 7) as for stage 1 and then press ENTER.

N	E	W						A	:	0	5		P	C	R		6	8	C
S	T	A	G	E		0	2				S	T	E	P		0	2		
T	E	M	P		1	0	:	0											
T	I	M	E		1	:	0	0	:	0	0								

11. Press ENTER (or ↓ key) with each parameter at zero to finish stage 2.

N	E	W						A	:	0	5		P	C	R		6	8	C
S	T	A	G	E		N	U	M	B	E	R						0	2	
N	U	M	B	E	R		O	F		C	Y	C	L	E	S		0	1	
H	O	L	D		T	E	M	P			0	0	.	0					

12. (Enter number of cycles of stage 2, and press ENTER.
Enter a hold temperature if required.
NOTE: During operation, hold temp is maintained until CONTINUE is pressed. Press ENTER to proceed.

N	E	W						A	:	0	5		P	C	R		6	8	C
S	T	A	G	E		0	3				S	T	E	P		0	1		
T	E	M	P		0	0	:	0											
T	I	M	E		0	:	0	0	:	0	0								

13. Press ENTER (or ↓ key) with each parameter at zero for step 1 of the final stage of programming.

S	A	V	E				A	:	0	5		P	C	R		6	8	C
<	E	N	T	E	R	>		T	O		S	A	V	E				
<	M	E	N	U	>			T	O		A	B	A	N	D	O	N	

5.5 Viewing an Existing Program

You can look at existing programs without altering the information contained within them using the VIEW command.

1. Select PROG from the Main Menu, VIEW from the program menu and (using the ← and → keys) enter the source directory.
2. Use the ENTER key and the ↑ and ↓ arrows to move through the program, and CONTINUE to enter and leave the Advanced Edit information.
3. The MENU key closes the view screen, and returns to the Main Menu.

5.6 Editing an Existing Program

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

To edit and overwrite an existing program select PROG from the Main Menu and EDIT from the program menu, and select EXISTING from the sub menu. Select and enter the source directory (using ← and →).

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

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

The ↓ and → arrow keys are used to advance through each stage, using the number keys to change each time and temperature set point as desired.

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

Leaving a step set to 0 for all parameters denotes the end of the cycling step. Leaving the first step in a stage at 0 for all parameters denotes the end of the program.

The number of cycles can then be added, and a hold temperature as required. Note that the hold temperature is defaulted to 00.0^{o}C, which denotes no hold step, and the thermal cycler will shut down. (See Section 4.7.)

Save program with edits by pressing ENTER at “save as” screen. Also save program without edits by pressing MENU at “save as” screen.

NOTE. Care needs to be taken when editing programs, which use Advanced Edit features in case undesired functions are inadvertently left in edited programs.

5.7 Copying Programs

Copying programs is necessary in, for example, modifying the Preset programs described in Appendix III. From the PROGRAM menu, select COPY and press ENTER.

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

5.8 Erasing a Program

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

From the PROGRAM menu, select ERASE and press ENTER.

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

FIGURE 5.2: Editing Programs on the PCR Sprint using the "EXISTING" Program Option

1. Move the cursor to PROG, then press ENTER to select.

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t	
						I	N	A	C	T	I	V	E						
						M	A	I	N		M	E	N	U					
R	U	N			M	A	N			P	R	O	G		S	E	T	U	P

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

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t	
						I	N	A	C	T	I	V	E						
				P	R	O	G	R	A	M		M	E	N	U				
E	D	I	T		C	O	P	Y		V	I	E	W		E	R	A	S	E

3. Press ENTER to select EXISTING program to edit.

		T	H	E	R	M	O		P	C	R		S	p	r	i	n	t	
						I	N	A	C	T	I	V	E						
P	R	O	G	R	A	M		E	D	I	T	I	N	G		M	E	N	U
E	X	I	S	T	I	N	G			N	E	W							

4. Use the ↑,↓ keys to select the source directory then press ENTER.

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

5. Select program number (01-50 ur use ↑,↓ keys). Press ENTER to select.

E	D	I	T				A	:	0	1		N	0		N	A	M	E
<	E	N	T	E	R	>		T	O		C	O	N	T	I	N	U	E

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

E	D	I	T				A	:	0	5		P	C	R		6	5	C
<	E	N	T	E	R	>		T	O		C	O	N	T	I	N	U	E

7. Use ➔ to move across menu, and ↓ or ENTER to advance through the data fields and from step to step, editing the time and temperature settings as required.

E	D	I	T				A	:	0	5		P	C	R		6	5	C
S	T	A	G	E		0	1				S	T	E	P		0	1	
T	E	M	P		9	4	:	0										
T	I	M	E		0	:	0	2	:	0	0							

8. Increase number of steps (up to 5) in a stage by writing data into zero temperature/time fields.
   Decrease number of steps in a stage by writing zero data into existing temperature/ time fields.

E	D	I	T				A	:	0	5		P	C	R		6	5	C
S	T	A	G	E		0	1				S	T	E	P		0	2	
T	E	M	P		0	0	:	0										
T	I	M	E		0	:	0	0	:	0	0							

9. Press ENTER (or ↓ key) with each parameter at zero to finish editing stage 1.

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

10. Enter new number of cycles for stage 1 and press ENTER, and any HOLD temperature change. Press ENTER to go to the next stage.

E	D	I	T					A	:	0	5		P	C	R		6	5	C
S	T	A	G	E		0	2				S	T	E	P		0	1		
T	E	M	P		9	4	:	0											
T	I	M	E		0	:	0	1	:	0	0								

11. Edit steps for further stages as required to a maximum of 5, as for stage 1, and then press ENTER.

E	D	I	T					A	:	0	5		P	C	R		6	5	C
S	T	A	G	E		0	4				S	T	E	P		0	1		
T	E	M	P		0	0	:	0											
T	I	M	E		0	:	0	0	:	0	0								

12. Press ENTER (or ê key) with each parameter at zero to step 1 of the final stage of programmingj to finish editing.

S	A	V	E					A	:	0	5		P	C	R		6	5	C
<	E	N	T	E	R	>		T	O		S	A	V	E					
<	M	E	N	U	>			T	O		R	E	T	A	I	N			
								O	L	D		P	R	O	G	R	A	M	

5.9 Advanced Edit Features

The Advanced Edit features allow creation of more complex cycling protocols in order to enhance experimental data. These include altering the rate of temperature change between temperature set points and the increment or decrement of both time and temperature on a cycle-by-cycle basis. Advanced Edit features are accessed by pressing the CONTINUE key at the temperature step at which the function is to take effect. If Advanced Edit data already exists for any step - ADV EDIT is indicated on the screen (see fig 5.3).

Enter and change Advanced Edit parameters using the number, arrow and ENTER keys in the same way as for the normal editing screens.

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 EDIT message will then not be shown in the temperature/time set-up screen, denoting that no ADV EDIT functions are operating.

Changing Ramp Rates (also see Fig 5.3)

The default ramp rate (0.00°C/sec) is "as fast as possible". The setting range is between0.01°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.

FIGURE 5.3 Advanced Edit

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

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

2. Press CONTINUE to access the Advanced Edit Screen.

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

3. Enter the desired ramp rate (^{o}C/second) using the number keys and press ENTER.

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

4. Move through the screen (making other additions to the functions as required) back to the main step screen using the ↓ key. Note that ADV EDIT has now appeared on the screen.

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

Temperature/Time Increment/Decrement

The Advanced Edit feature allows increment and decrement of both time and temperature on a cycle-by-cycle basis. The user can specify the increment/ decrement per cycle for each program stage.

This feature has applications where experimental data can be enhanced by the use of more complex cycling procedures.

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

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

The Advanced Edit feature is accessed from the normal EDIT program menu options. Examples are given below:

Time Advanced Edit Worked Example

A protocol where the extension step is fixed for the first 15 cycles of a 25 cycle program and increasing 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	

FIGURE 5.4 Programming Time Increments

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

E	D	I	T	I	N	G		A	:	0	6		P	C	R	@	5	5	C
S	T	A	G	E		0	2				S	T	E	P		0	3		
T	E	M	P		7	2	:	0											
T	I	M	E		0	:	0	0	:	3	0								

2. Press CONTINUE to access the Advanced Edit Screen.

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

3. Move through the screen to the time field (making additions to the RAMP function as required) using the 🡻 key.
   Use 🡺 to toggle between Increase and Decrease in set point time/cycle.

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

4. Enter the desired amount by which the set point time per cycle should change (mins:seconds) using the number keys.

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

5. Move through the screen (making additions to the TEMP function as required) back to the main step screen using the 🡻 key.
   Note that ADV EDIT has now appeared on the screen.

E	D	I	T	I	N	G		A	:	0	6		P	C	R	@	5	5	C
S	T	A	G	E		0	2				S	T	E	P		0	3		
T	E	M	P		7	2	:	0									A	D	V
T	I	M	E		0	:	0	0	:	3	0					E	D	I	T

Temperature Advanced Edit Worked Example

A protocol where the annealing step is fixed for the first 5 cycles of a 25 cycle program and decreasing by 0.2°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 - 0.2°C/cycle	x 16 Cycles
72°C - 30s	

Stage 3: Enter parameters to create:

95°C - 30s	
62°C - 30s	x 5 Cycles
72°C - 30s	

FIGURE 5.5 Programming Temperature Decrements

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

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

2. Press CONTINUE to access the Advanced Ed it Screen.

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

3. Move through the screen to the TEMP field (making additions to the other functions as required) using the 🡻 key. Use 🡺 to toggle between Increase and Decrease in set point temp/cycle.

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

4. Enter the desired amount by which the set point temp per cycle should change (°C) using the number keys.

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

5. Move back to the main step screen using the 🡻 key. Note that ADV EDIT has now appeared on the screen.

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

5.10 Achieving Optimum Results with PCR Sprint

Check volume in the tube thermistor

Check the volume of mineral oil in the control tube (a vial of suitable mineral oil is provided or Sigma molecular biology grade mineral oil M5904 or equivalent can be used).

- Do not use aqueous solutions in the control tube.
- Too much oil in the control tube will result in overshoots in the sample tube temperatures.
- Too little oil there will be 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 gradually reduce the activity of the thermostable enzyme resulting in poor yields of product. The correct position for the control tube is in 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 it is pushed against the side it will be measuring the temperature of the microcentrifuge tube and not the sample temperature.

Check the fit of the tube in the block

The PCR Sprint block accepts most types of reaction tubes. The tube thermistor supplied with the PCR Sprint is mounted in an appropriate OmniTube, and this tube should be changed periodically, as the fit of the tube will deteriorate with time. The reaction tubes should be distributed evenly in the block. If you have opted to use a reaction tube other than one of those recommended, check its fit in the PCR Sprint block before use. Also remount the thermistor probe in this tube type to ensure your samples and the control tube is well matched.

Check through the program before running using the VIEW function. Have you entered the correct combination of program number?

Runs using Simulated Control methods

For reactions in tubes, enter the number of samples including the thermistor, and the total reaction volume per tube including any oil overlay. Add 50µl to the volume factor if thick walled tubes are used.

Check maximum and minimum values achieved during the run, which can give an indication of unusual temperature performance.

Power failure during a run

This will be indicated by a message 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 stage, step and cycle of the power failure.

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

Always consider the different modes of control before transferring protocols directly. As an example, 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 results 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 direct 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 obtain a thermistor lead extension from Thermo Electron Corporation and use it with the control tube of the PCR Sprint as a temperature probe. This is used to monitor the block control machine as follows:

1. Set the PCR Sprint to run an arbitrary program (e.g. F:51) using Block Control.
2. Connect the thermistor lead extension to the thermistor control tube and plug the other end into the PCR Sprint.
3. Locate the thermistor tube probe into a well of the block control machine running the required protocol.
4. After a short equilibration interval, the "Tube" display on the PCR Sprint will indicate the sample tube temperature, which should be monitored over a number of cycles. In particular, the actual length of time spent at each of the denaturation, annealing and elongation stages should be recorded, as well as any temperature overshoot values where the maximum/minimum temperature exceeds the target temperature or undershoots where the target temperature is not actually reached.
5. The temperature profile that the samples in the block control machine actually achieve, rather than simply the block temperature, can be used to program your PCR Sprint.

5.11 Optimization of protocols

In addition to the differences between tube control and block control programs noted above, it is important to optimize the protocol itself. The flexibility of the programming capability of the PCR Sprint enables this to be performed very rapidly. Typically, temperature cycling protocols may consist of three distinct stages:

1. Denaturation at an elevated temperature (usually 90-95°C).
2. Annealing at a temperature dictated by the melting temperature (Tm) of the oligonucleotides.
3. Enzymatic activity at a temperature dictated by the optimum temperature of the thermostable enzyme being used.

These three steps are typically repeated for twenty to thirty cycles depending on the amount of starting template. 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 serve to 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. Optimization of the denaturation step is the most critical factor when transferring a protocol from a block control machine to tube control.

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, an approximation for which may be calculated using the formula:
$Tm = 2 \times (A + T) + 4 \times (G + C)$

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

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 synthesized. A time of one minute per kilobase should be more than sufficient to begin with. Some protocols (termed 'biphasic') dispense with a separate extension step altogether. These rely on the activity of the enzyme at the annealing temperature, and during the transition from the annealing temperature to the denaturation temperature, being sufficient to synthesize the amplification product.

6. Comparative Performance Data

Although there are fundamental differences between the methods of heating and cooling for these different block systems, in the majority of cases direct transfer of protocols will be possible. This is because the temperature and time control system is similar for the PCR Sprint, PCR Express, TouchDown, Omn-E and OmniGene systems, even though the performance and accuracy characteristics are different. There are some cases however, when the biological performance of the two instruments for a given cycling protocol will be different. One obvious example is for reactions with annealing temperatures close to ambient. Less obvious is the effect of default ramp rates on other stages of the reaction (see Table 6.1).

Table 6.1: Block Ramping Characteristics of Ambient and Sub-ambient Units

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

7. Maintenance, Radiochemical and Electrical Safety

7.1 General Cleaning

Before using any cleaning or decontamination method except those recommended below, please contact technical service an authorized dealer to check that the proposed method will not damage the equipment.

All surfaces of the PCR Sprint system and heated lid should be cleaned regularly with a soft cloth, hot water and a mild detergent. It is important to thoroughly dry all surfaces after cleaning. The PCR Sprint is not intended for use with aggressive chemicals and on no account should organic solvents be used in the cleaning of any of this equipment.

Dampened cotton applicators can be used to remove dirt and debris from individual wells as required. Keeping the wells clean is important to maintain optimum heat transfer performance.

7.2 Decontamination

It has been observed by researchers that when 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 do not recommend the use of ^{35}S labels, as replacing a contaminated block is expensive.
- This is not covered under our warranty agreement and requires special service arrangements.
- If ^{35}S labels must be used, the following recommendations will assist in minimising contamination.
- Use a mineral oil overlay in all reactions, even if using the heated lid.
- If using tubes, use only the thick walled variety.
- Use the thermal cycler in a fume hood, as air contamination is a possibility.

All Control Chassis, Block Module, and Heated Lid components, which may come into contact with radioactivity, should be decontaminated before re-use or transportation. Any contaminated item requiring Thermo Service Department attention should be notified to the Service Department for appropriate handling arrangements to be made.

If radioactivity must be used, the thermal cycling block and Heated Lid surfaces can be decontaminated using a 10 % v/v solution of Neutracon (Decon Lab Ltd, Conway Street Hove, East Sussex BN3 3LY Tel: +44(0)1273-739241, Fax: +44(0)1273-722088 or PCC-54 (Pierce Eurochemie B.V Holland). It is important to stress that 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.

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.

7.3 Protection to the User

The PCR Sprint 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 be corrupted or bypassed, electrical circuitry is in place to ensure that safe temperatures are not exceeded. In the event of this being compromised, thermal fuses are fitted to shut off the power supply to damaged components.

7.4 Protection of the Instrument

Fuses

The PCR Sprint mains power inlet is fitted with two T2.0A fuses (20mm x 5mm) to protect the unit from electrical damage. If necessary these may be replaced with proprietary equivalents, which should be fitted by an appropriately qualified person.

8. Specifications & Ordering Information

Description	PCR Sprint with 0.5ml Block	PCR Sprint with 0.2ml Block
Catalogue Number	HBSP05110 (110 Volt) HBSP05220 (220 Volt)	HBSP02110 (110 Volt) HBSP02220 (220 Volt)
Block Capacity	20 x 0.5ml tubes	24 x 0.2ml tubes
Temperature Control Available	Tube control Simulated tube control Block control	Tube control Simulated tube 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
Thermistor Precision	± 0.1°C	± 0.1°C
Block Uniformity	± 0.5°C within 15 secs	± 0.5°C within 15 secs
Heated Lid Temperature	115°C max	115°C max
Display Resolution	0.1°C	0.1°C
Ingress Protection Rating	20	20
Standard Accessories	Tube Thermistor 0.5ml (HB-PX-TTM05)	Tube Thermistor 0.2ml (HB-PX-TTM02)
Interchangeable Block Modules	HB-SP-B05	HB-SP-B02

PROGRAMMING (all unit variants)	
Number of programs	60
Number of directories	6
Maximum number of program stages	5
Maximum number of steps per stage	5
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
Oil free operation	Yes
Alphanumeric program naming	Yes
Ability to edit during cycle	Yes
Power	250W
Dimensions (W x D x H)	180mm x 300mm x 230mm
Weight	7.5Kg

Specifications are subject to change without notice.

Working Conditions

- Ambient Temperatures of 4°C to 35°C
- Power requirements: 250W at 115/230V a.c. ±10% and 50/60Hz.

NOTE:When the PCR Sprint Temperature Cycler is removed from the cold room, it must first be left to equilibrate for 2-3 hours to avoid condensation

Designed to meet the requirements of IEC 1010-1:1995 installation category 2, pollution degree 2.

Designed to meet the electrical safety standards as described in IEC 1010-1:1995 requirements.

The PCR Sprint is a class 1 (Grounded) appliance. It must be connected to a protected earth connection via the supplied mains cord.

9. APPENDIX 1: Quick Reference Guide

An alphabetical summary of some of the terms used in the programming and operation of the PCR Sprint.

Advanced Edit	Allows: 1) Incrementation / decrementation of time and/or temperature on a cycle-by-cycle basis. 2) Control of temperature ramping (ramp rate) between set points. (See Section 5.9.)
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 reaching temperature and the sample reaching temperature it is recommended that tube control be normally used. (See Section 4.2.)
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. (See Section 5.7.)
Edit	Edit is used to create new programs or to change existing programs. (See Section 5.6.)
Erase	Erase is used to delete programs/create extra space for new protocols. (See also Section 5.8.)
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. Useful to hold samples at a fixed temperature while a reagent is added before temperature cycling starts. (See Section 4.5.)
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. (See Section 3.6.)
Menu	The menu key allows user to exit higher levels of the programming and return to the main menu without making any changes in the programming/ operation of the unit.
New Temperature	A new temperature may be specified during a manual incubation step. The manual program is interrupted and the sample temperature rapidly changes to the newly specified temperature.
Peltier Device	A thermoelectric device incorporating a bismuth telluride semi-conductor crystal array. On passing a current through the crystal, one surface of the crystal is heated, the other cooled. Reversing the current reverses the heat flow.
Power Failure	The PCR Sprint 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 power failure step. 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 to create a new program, or to copy, view or edit a previously stored program (see section 5: Programming the PCR Sprint).
Ramp Rate (Temperature Ramping)	Ramping precisely controls the rate of change of sample temperature (°C/ sec). Useful for limiting the rate of change of temperature to allow partial extension of short or degenerate primers, for example.

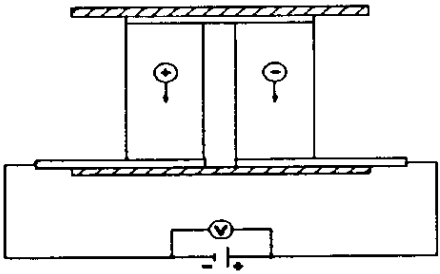
RUN	The RUN Option allows the user to run a stored program on the block (see section 4).
Sample Volume	When using simulated tube control methods, the sample volume (including oil overlay unless oil free) is entered to ensure precise block heating and cooling. (See Section 4.2.)
SET UP	This function allows the default settings of the PCR Sprint (block type and power restart option) to be modified. (See Section 3.4: Set Up Functions.)
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. Refer to Section 4.2 for instructions on how to use this control option.
Stage	One stage of a program consists of one or more steps up to a maximum of 5. A stage may be repeated for temperature cycling. Up to seven stages may be used in one program, and stage-to-stage linking is automatic.
Step	A step consists of a programmed temperature and time interval. Time increment and ramp rate may also be specified if required. Enter zero for each heading to specify the last step of a particular stage, and enter zero for each heading at the first step of the final stage.
Sub-ambient Block	An aluminium block is used on sub-ambient PCR Sprint systems. This uses Peltier devices for heating and cooling. Operating range: 4°C to 99°C.
TEMP INC/DEC Temperature Increment/ Decrement	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, and 59.7°C. 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. Useful to increase incubation step to allow for depletion of an enzyme, for example.
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 and 0.5ml tubes. The presence of a height adjusting heated plate in contact with the top of the reaction vessels maintains the temperature above 100°C, preventing evaporation of the sample.
VIEW	The View function allows the contents of programs to be accessed without risk of accidental editing. (See Section 5.5.)

10. APPENDIX 2: Peltier Technology

The Peltier effect heat pump, also known as a thermoelectric device, is the key component within the Thermo Electron PCR Sprint Sub-ambient unit. Positioned between the sample block and the heat sink, the heat pump is the means by which the block temperature is controlled.

Peltier devices depend on the discovery that passing an electric current through the junction of two dissimilar conductors coupled together can either cool or heat the junction, depending on the direction of the current. Reversing the current reverses the direction of the heat transfer. The semiconductor Bismuth telluride exhibits the best heat pumping characteristics in the temperature range for thermal cycling operations.

In Thermo units, n-type Bismuth telluride and p-type Bismuth telluride ("doped" during manufacture with an excess and a lack of electrons respectively) are arranged in a matrix. Coupled electrically in series, but thermally in parallel, these produce the best possible heating and cooling characteristics.



Until recently, Peltier devices had proven unreliable as a temperature control method for thermal cycling. Now, changed manufacturing methods have allowed the development of a robust, high quality custom product using proven technology. In combination with proprietary, sophisticated control software, this system gives a thermal cycler of outstanding speed, uniformity and accuracy.

11. APPENDIX 3: Pre-Set Programs

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

PROGRAM F:51	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:52	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:53	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
PROGRAM F:54	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:55	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
	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

PROGRAM F:56	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:57	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:58	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
	Step 2	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
PROGRAM F:59	DIGEST			
Stage 1	Step 1	37°C	4:00:00	x 1
PROGRAM F:60	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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