PTC-220 DNA Engine Dyad™ Peltier Thermal Cycler

Operations Manual

Version 1.3

Boston • San Francisco • Tahoe • Copenhagen • Seoul

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Licensing Manager
Roche Molecular Systems, Inc.
1145 Atlantic Avenue
Alameda, CA 94501 USA
(510) 814-2970
Fax: (510) 814-2977

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PCR Licensing Manager
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Fax: 41-61-687-2113

Contents

Documentation Conventions

Before describing the various features of the Dyad cycler, let's define some "common ground" conventions.

- << >> will be used to indicate actual keys on the control panel, such as <<ENTER>>, <<1>> and <<LEFT>>.

- < > will be used to indicate windowed menu items or buttons, such as <PROGRAMS>, <RUN> and <TOOLS>.

- *Italics* will be used to indicate windowed items that are not drop down menu items or buttons, such as *Calculated*, *Block*, and *Tracking*. Typically, these will be parameter selection items.

- ***Select*** is meant to be synonymous with ***click on***, ***point-and-click***, and any phraseology implying selection of menu or option items with a mouse. Particularly with the Dyad cycler, ***select*** should symbolize any physical selection on the Dyad input devices (touch pad/mouse, numeric keypad, arrow keys) to access one of the user interface windows. This includes single or double taps on the touch pad, pressing of the left touch pad button, or pressing the <<Enter>> button on the numeric keypad.

1 Introduction

Meet the DNA Engine Dyad Cycler

Thank you for purchasing an MJ Research DNA Engine Dyad™ thermal cycler. Designed by a team of molecular biologists and engineers, the DNA Engine Dyad cycler delivers multi-block thermal cycling with superior thermal performance. The programmable Dyad™ cycler, with its dual-bay chassis, is ideal for running multiple protocols and accommodating multiple users. Some of the Dyad cycler's many features include:

- Interchangeable sample blocks—the Alpha™ unit family accommodates a variety of tubes, microplates, and slides
- Hot Bonnet™ heated lid for oil-free cycling or the Power Bonnet™ lid for automated systems
- Intuitive software with user-friendly interface for programming, editing, file management, and much more
- Choice of calculated temperature control for highest speed and accuracy, or of block or probe temperature control for compatibility with protocols designed for a variety of instrument types
- Instant Incubate feature for continuous-temperature incubations

Using This Manual

This manual contains instructions for operating your DNA Engine Dyad cycler safely and productively:

- Chapter 2 acquaints you with the **physical characteristics** of the DNA Engine Dyad cycler.
- Chapters 3–4 present the basics of **installation and operation** for the DNA Engine Dyad cycler.
- Chapters 5, 6 and 7 describe the **creation, editing and running of programs**.
- Chapter 8 outlines the software **utilities**.
- Chapter 9 explains the proper **maintenance** of the DNA Engine Dyad cycler.
- Chapter 10 offers **troubleshooting** information for the DNA Engine Dyad cycler.
- Chapter 11 describes the **installation and operation of the RAD-200 Remote Alpha Dock™ accessory**.

Important Safety Information

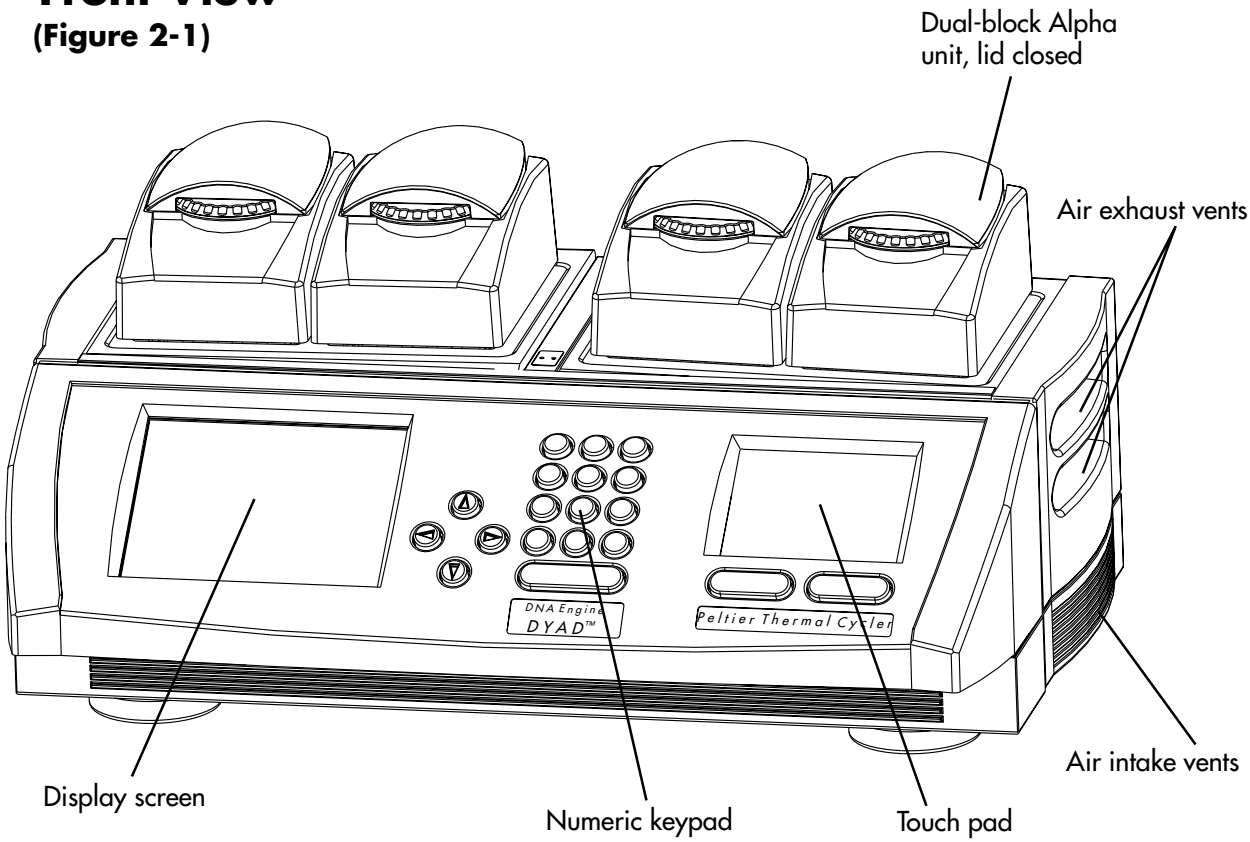
Safe operation of the DNA Engine Dyad cycler begins with a complete understanding of how the instrument works. Please read this entire manual before attempting to operate the Dyad cycler. Do not allow anyone who has not read this manual to operate the instrument.

⚠ **Warning:** The DNA Engine Dyad cycler can generate enough heat to inflict serious burns and can deliver strong electrical shocks if not used according to the instructions in this manual. Please read the safety warnings and guidelines in Appendix A, and exercise all precautions outlined in them.

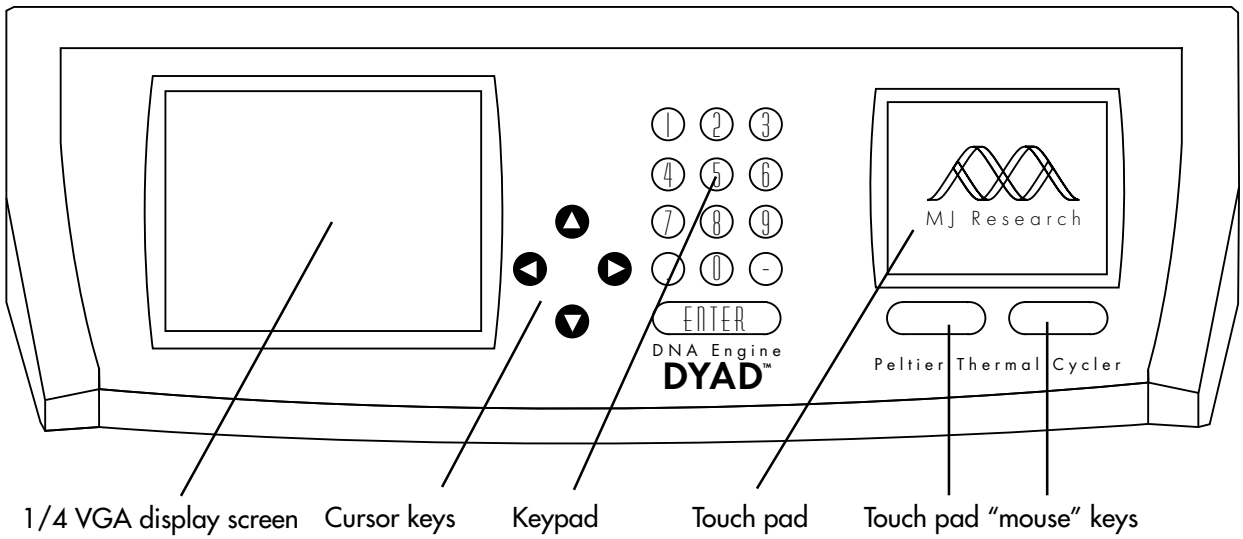
⚠ **Warning:** Do not block the Dyad cycler's air vents (see figs. 2-1 and 2-4 for location). Obstructing air vents can lead to overheating and slightly enhanced risk of electrical shock and fire.

2 Layout and Specifications

Front View
(Figure 2-1)

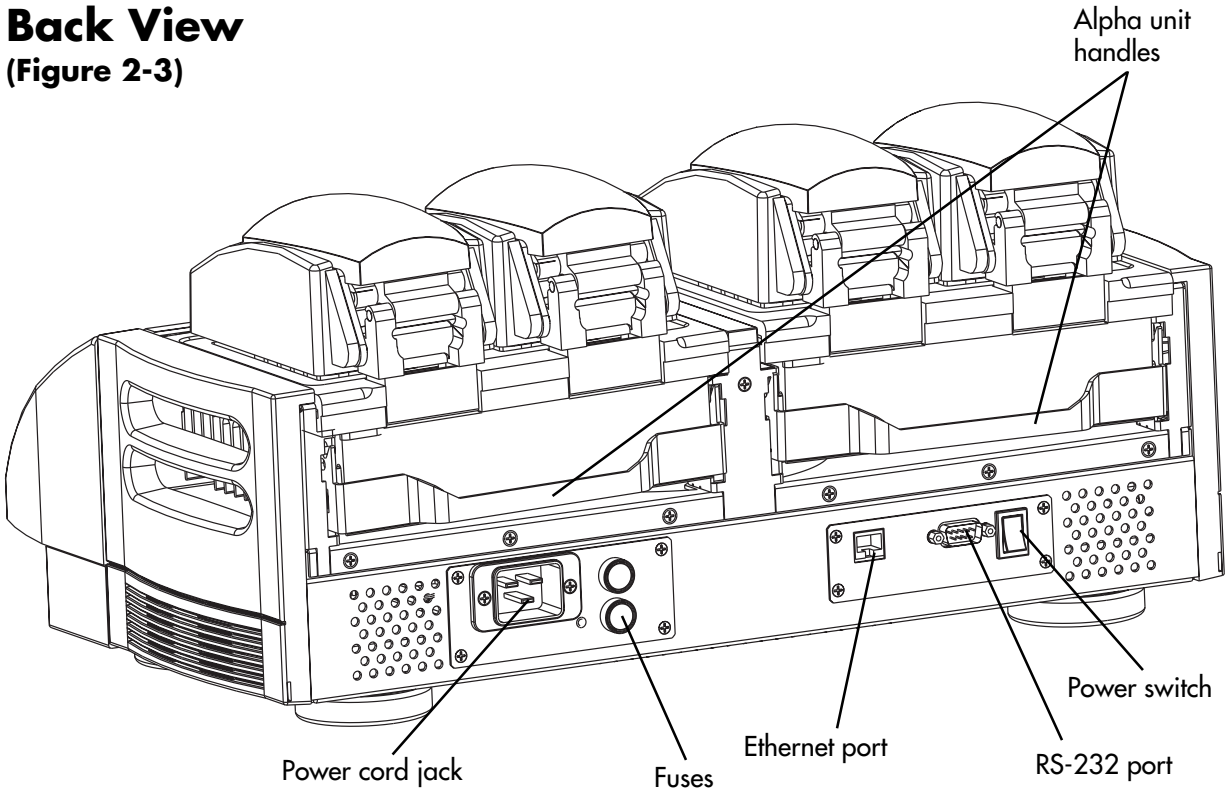


Control Panel
(Figure 2-2)



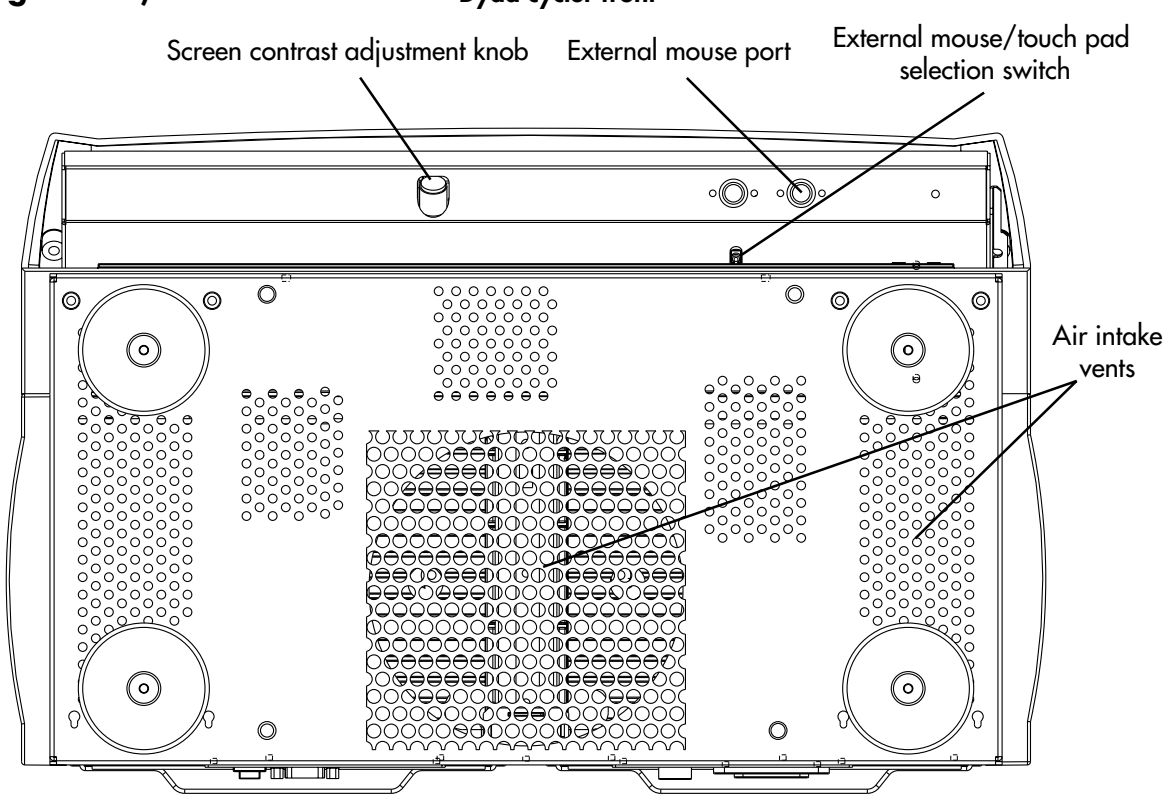
Back View

(Figure 2-3)



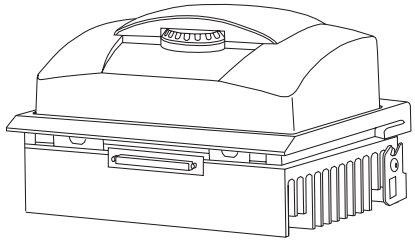
Bottom View

(Figure 2-4)



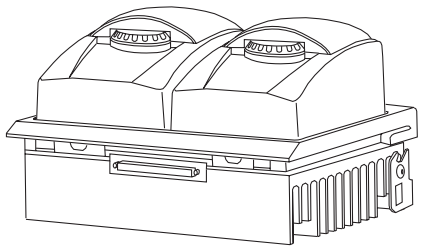
Alpha™ Units

Single-Block Models



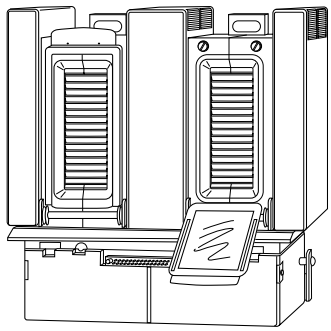
60 Single:	Holds 60 x 0.5ml tubes
96 Single:	Holds 96 x 0.2ml tubes or one 96-well microplate
384 Single:	Holds one 384-well microplate
Flat Block:	Holds customer-designed adapter through four screw-down points

Dual-Block Models



30/30 Dual:	Holds 2 x 30 x 0.5ml tubes
30/48 Dual:	Holds 1 x 30 x 0.5ml tubes and 1 x 48 x 0.2ml tubes
48/48 Dual:	Holds 2 x 48 x 0.2ml tubes or half plates

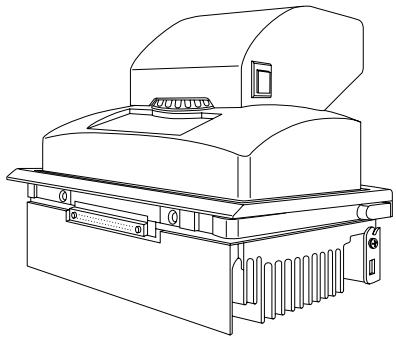
Slide Block



Twin Towers:	Holds 2 x 16 standard slides

Power Bonnet Accessory

Permits remote control of Alpha unit lid opening; available for Alpha unit models 96, 384, and flat block.



Specifications

Thermal range:	–5.0° to 105°C, but no more than 30°C below ambient temperature (4°C to 105°C, but not more than 23°C below ambient temperature for the Twin Towers® unit)
Accuracy:	± 0.3°C of programmed target @ 90°C, NIST-traceable
Thermal uniformity:	± 0.4°C well-to-well within 30 seconds of arrival at 90°C (for most Alpha units; see specifications for individual Alpha units)
Ramping speed:	Up to 3°C/sec for all single- and dual-block Alpha units; Up to 1.2°C/sec for the Twin Towers® unit
Sample capacity:	Varies with installed Alpha unit
Line voltage:	200-240VAC
Frequency:	50-60Hz
Power:	1600W maximum
Fuses:	Two 6.3A, 250V, 5 x 20mm
Displays:	One 1/4 size VGA screen (320x240), 16 colors
Ports:	One 9-pin RS-232 serial port One ethernet port
Memory:	8 MB
Weight:	11kg (base only)
Size:	48 x 29 x 15cm (l x w x h, base only)

Gradient Specifications (96 Alpha unit only)

Accuracy:	± 0.4°C of programmed target at end columns, 30 seconds after the timer starts for the gradient step, NIST–traceable
Column uniformity:	± 0.4°C, well–to–well within column, within 30 seconds of reaching target temperature
Calculator accuracy:	± 0.4°C of actual well temperature
Lowest programmable temperature:	30°C
Highest programmable temperature:	105°C
Temperature differential range for gradient:	1–24°C

3 Installation

Packing Checklist

After unpacking the DNA Engine Dyad cycler, check to see that you have received the following:

- One DNA Engine Dyad base
- Two spare fuses
- One power cord
- One external mouse device
- *PTC-220 DNA Engine Dyad Peltier Thermal Cycler Operations Manual* (this document)
- Product registration card (US & Canada only)
- Extended warranty application (US & Canada only)

If any of these components are missing or damaged, contact MJ Research or the authorized distributor from whom you purchased the DNA Engine Dyad cycler to obtain a replacement. Please save the original packing materials in case you need to return the Dyad cycler for service. See Appendix C for shipping instructions.

Setting Up the DNA Engine Dyad Cycler

The DNA Engine Dyad cycler requires only minimal assembly, plugging in the power cord and mounting the Alpha units. Insert the power cord plug into its jack at the back of the machine (see figure 2-3 for location of jack), then plug the cord into a 220V electrical outlet. With the machine switched off, mount the Alpha units (see the "Installing an Alpha unit" section in Chapter 4).

⚠ **Caution:** Do not insert or remove an Alpha unit with the Dyad cycler turned on; electrical arcing can result. Read the safety warning in Appendix A regarding electrical safety when inserting or removing an Alpha unit.

Optional External Mouse Device

Included with each shipment of a DNA Engine Dyad™ thermal cycler is an externally attachable mouse, intended to substitute for the function of the touch pad. Should a Dyad user prefer an externally attached mouse device, rather than the integrated touch pad, the mouse should be attached prior to power up of the Dyad cycler.

Underneath the front lip of the Dyad cycler, positioned at the mid-point of the touch pad, are two connection ports (see figure 2-4). The purple port on the left is reserved for future function and should not be used. The green port on the right is for connecting the external mouse.

To insure complete compliance with FCC and EMC requirements, only a mouse with a ferrite core should be used with the Dyad instrument.

To connect the mouse, please follow these steps:

1. Verify that the Dyad cycler is off. Wait for 10 seconds to ensure that all fans have stopped rotating.
2. Grasping the sides of the Dyad cycler, tilt the instrument back so that the underside of the lip is visible.
3. Line up the pins of the mouse connector with the green port and push the connector into place.
4. Pull the small switch located behind the purple port into the forward position (see figure 2-4). The rear position will activate only the touch pad. The forward position will activate only the external mouse device. All Dyad cyclers are shipped with the touch pad enabled (i.e., the switch is in the rear position). Please note that the cycler will recognize either the touch pad OR a mouse, but not both input devices simultaneously.
5. Tip the Dyad cycler back down and power up the system.

Environmental Requirements

Ensure that the area where the DNA Engine Dyad cycler is installed meets the following conditions, for reasons of safety and performance:

- Nonexplosive environment
- Normal air pressure (altitude below 3000m)
- Ambient temperature 5˚–31˚C
- Relative humidity of 10–90% (noncondensing)
- Unobstructed access to air that is 31˚C or cooler (see below)
- Protection from excessive heat and accidental spills. (Do not place the Dyad cycler near such heat sources as radiators, and protect it from danger of having water or other fluids splashed on it, which can cause shorting of its electrical circuits.)

Power Supply Requirements

The DNA Engine Dyad cycler requires 200-240VAC, 50-60Hz, and a grounded outlet on a minimum 20A line. The Dyad cycler can use voltage in the specified range without adjustment, so there is no voltage-setting switch.

Note: Do not cut the supplied power cord and attach a different connector. Use a one-piece molded connector. If required, additional dedicated power cords may be purchased through MJ Research or authorized distributors.

Air Supply Requirements

The DNA Engine Dyad cycler requires a constant supply of air that is 31°C or cooler in order to remove heat from the Alpha unit's heat sink. Air is taken in from vents at the bottom and sides of the machine and exhausted from vents on both sides (see figures 2-1, 2-3, and 2-4). If the air supply is inadequate or too warm, the machine can overheat, causing performance problems, software error messages (particularly "HS Overheating" and "Slow Block Cycling"), and even automatic shutdowns. Special attention should be paid to airflow and air temperature in robotics installations of DNA Engine Dyad cyclers.

Ensuring an Adequate Air Supply

- Do not block the air-intake vents.

  Position the DNA Engine Dyad cycler at least 10cm from vertical surfaces and other thermal cyclers (greater distances may be required; see below). Do not put loose papers, bench paper, or this manual under the instrument; they can be sucked into the air-intake vents on the bottom.

- Do not allow dust or debris to collect in the air-intake vents.

  The bottom air vents are particularly liable to collect dust and debris, sometimes completely clogging up. Check for dust and debris every few months, and clean the intake vents as needed. Remove light collections of dust with a soft-bristle brush or damp cloth. Severe collections of dust and debris should be vacuumed out. Turn the instrument off prior to cleaning or vacuuming air vents.

- Use a solid, non-perforated support material when using the Dyad cycler on a wire rack.

Ensuring That Air Is Cool Enough

- Do not position two or more DNA Engine Dyad cyclers (or other thermal cyclers) so that the hot exhaust air of one blows directly into the air-intake vents of another.

- Make sure the DNA Engine Dyad cycler receives air that is 31°C or cooler by measuring the temperature of air entering the machine through its air-intake vents.

  Place the DNA Engine Dyad cycler where you plan to use it, and turn it on. Try to reproduce what will be typical operating conditions for the machine in that location, particularly any heat-producing factors (e.g., nearby equipment running, window blinds open, lights on). Run a typical protocol for 30 minutes to warm up the DNA Engine Dyad cycler, then measure the air temperature at the back air-intake vents. If more than one machine is involved, measure the air temperature for each. If the air-intake temperature of any machine is warmer than 31°C, use Table 3-1 to troubleshoot the problem. Some experimentation may be required to determine the best solution when more than one cause is involved. After taking steps to solve the problem, verify that the temperature of the air entering the air-intake vents has been lowered, using the procedure outlined above.

Table 3-1 Troubleshooting Air Supply Problems

Cause	Possible Remedies
Air circulation is poor.	Provide more space around machine or adjust room ventilation.
Ambient air temperature is high.	Adjust air conditioning to lower ambient air temperature.
Machine is in warm part of room.	Move machine away from, or protect machine from, such heat sources as radiators, heaters, other equipment, or bright sunlight.
Machines are crowded.	Arrange machines so that warm exhaust air does not enter intake vents.

Requirements for Robotics Installations

Robotics installations require special attention to airflow and air temperature. Typically in these installations, DNA Engine Dyad cyclers and other thermal cyclers are restricted to a small area, along with other heat-generating equipment. Overheating can quickly occur when many of these instruments are operating at once, unless preventive measures are taken.

Follow the procedures described above to ensure adequate airflow and an air-intake temperature of 31°C or cooler. Air-intake temperature must be verified by measurement.

Do not use oil or glycerin to thermally couple sample vessels to the blocks of machines in a robotics installation. This can make plates difficult to remove.

384-Well Microplate Specifics

Some users find that a 384-well microplate can be difficult to remove from the 384-well block after completing their thermal cycling protocol. The plate fits very snugly in the block, and the 384 points of contact can provide a significant amount of friction. Fortunately, it is relatively simple to ameliorate this problem if it occurs in your application.

In our experience, a very thin coating of a Teflon®-based dry lubricant sprayed onto the block will solve the sticking problem very effectively. The coating eventually wears off so the block should be re-coated as needed, probably about once every 10 to 20 runs. Your experience will be the best guide in establishing the frequency for re-coating. As you will see, a very thin coat is sufficient to eliminate any sticking.

TFE (tetra-fluoroethylene) dry lubricant is available from many sources. One source in the United States is:

Miller-Stephenson Chemical Co., Inc.
in Danbury, CT: 203-743-4447
in Morton Grove, IL: 847-966-2022
in Sylmar, CA: 818-896-4714

TFE Dry Lubricant/Release Agent
Cat.# MS-122DF (aerosol, 10oz can) approx. $10.50/can

Here are some guidelines for applying the TFE lubricant.

1. Cool the block and lid to room temperature (below 38°C).
2. Cover the lid and any other areas that you don't want to get slippery.
3. Shake the can well.
4. Spray for about 1 second onto the block.

4 Operation

Turning the DNA Engine Dyad Cycler On

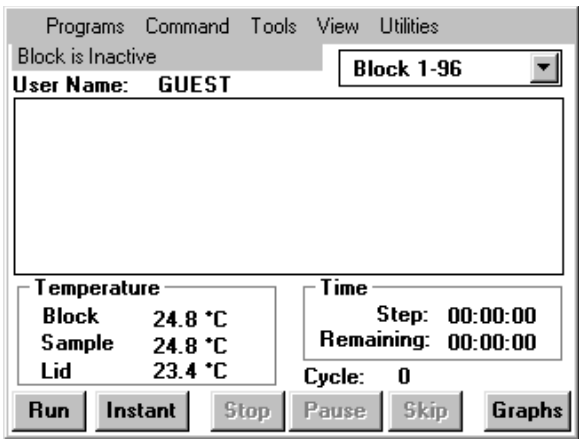
⚠ **Caution:** Do not insert or remove an Alpha unit with the DNA Engine Dyad cycler turned on; electrical arcing can result. Read the safety warnings in Appendix A regarding electrical safety before inserting or removing an Alpha unit or operating the Dyad cycler.

The Alpha units must be installed prior to Dyad cycler power up (see the "Operating Alpha Units" section below for installation instructions). The power switch is located at the back of the instrument (see figure 2-3). Turn the power switch on. The fan will turn on, the display screen will illuminate, and the microprocessor will implement a boot-up protocol lasting about 10 seconds. During the boot sequence, the user is presented with several options including:

1. **Selftest:** choose the number 1 on the numeric keypad to instruct the Dyad cycler to perform a diagnostic system test and report any errors.
2. **Send Files:** choose the number 2 on the numeric keypad to prepare the Dyad cycler to transfer stored program files to another Dyad cycler (see Chapter 8 for instructions on transferring program files).
3. **Receive Files:** choose the number 3 on the numeric keypad to prepare the Dyad cycler to receive stored program files from another Dyad cycler (see Chapter 8 for instructions on transferring program files).

If no option is selected, the boot sequence will automatically exit after approximately six seconds.

Following boot-up, the Dyad logo screen is briefly displayed. The Dyad Status window will then be visible. The DNA Engine Dyad cycler is now ready to accept, edit, and execute programs.



Using the Control Panel

The control panel (see figure 2-2) includes: a display screen, cursor keys, a numeric keypad with enter key, and a touch pad with left and right "mouse" buttons.

Display Screen

- The display screen is a 1/4 size VGA screen for displaying thermal cycler conditions and programs.

  Display screen contrast adjustment

  Underneath the front lip of the Dyad cycler, positioned below the cursor buttons, there is a small, partially recessed knob (see figure 2-4). This knob can be rotated to optimize the contrast of the color display.

Operation Keys

- **Cursor keys (left, right, up and down arrows):** Use to move around within the display screen.
- **Numeric keypad:** Use to enter numeric values.
- **Enter key (below keypad):** Use to accept specific programming additions and modifications.
- **Touch pad:** Use to move the display screen pointer with the movement of a finger tip. Once the pointer is positioned over a menu or selection item on the display screen, a single or double tap of the touch pad with a fingertip will implement the command. A tap on the touch pad corresponds to clicking the left button on a mouse.
- **'Mouse' buttons (left and right buttons below touch pad):** Pressing the left button is identical to a single tap on the touch pad or clicking the left button of a mouse.

  The right button has no current function.

Block Status Lights

- When illuminated, these blue lights indicate whether the left and/or right Alpha units are in use.

Using the Data Ports

The DNA Engine Dyad cycler has two data ports located at the rear of the machine: an RS-232 port and an Ethernet port. See Chapter 8 for information on using these ports.

Operating Alpha Units

Note: Operation of the Twin Towers® unit will not be discussed, owing to the many differences between this type of Alpha unit and the others. Please see the *Twin Towers Block Operations Manual* for operating instructions.

Note: Alpha units equipped with Power Bonnet™ lids are installed and removed as described below. See the *Power Bonnet Lid User's Manual* and the "Entering a Lid Control Step" section in Chapter 5 for information on opening and closing Alpha units with Power Bonnet lids.

Installing an Alpha Unit

⚠ **Caution:** Do not insert or remove an Alpha unit with the DNA Engine Dyad cycler turned on; electrical arcing can result. Read the safety warning in Appendix A regarding electrical safety when inserting or removing an Alpha unit.

1. **Turn the DNA Engine Dyad cycler off (see the Caution above).**
2. Hold the Alpha unit at its front and back edges.
3. Lower the Alpha unit into the DNA Engine Dyad base, leaving at least 3cm between the front edge of the Alpha unit and the front of the base.
4. Raise the handle at the back of the Alpha unit, and slide the block forward as far as it will go (see figure 4-1A).
5. Push the handle down until it is completely vertical (see figure 4-1B); firm pressure may be required, but do not force the handle into position. A definite click signals that the Alpha unit's connectors have mated with the DNA Engine Dyad cycler's connectors.

When the handle is in the down position, the Alpha unit is locked into place.

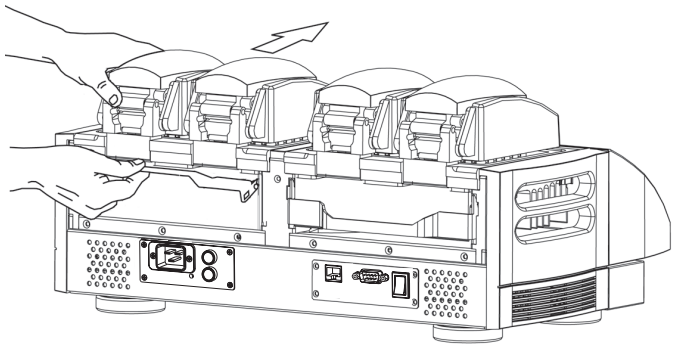
Removing an Alpha Unit

⚠ **Caution:** Do not insert or remove an Alpha unit with the DNA Engine Dyad cycler turned on; electrical arcing can result. Read the safety warning in Appendix A regarding electrical safety when inserting or removing an Alpha unit.

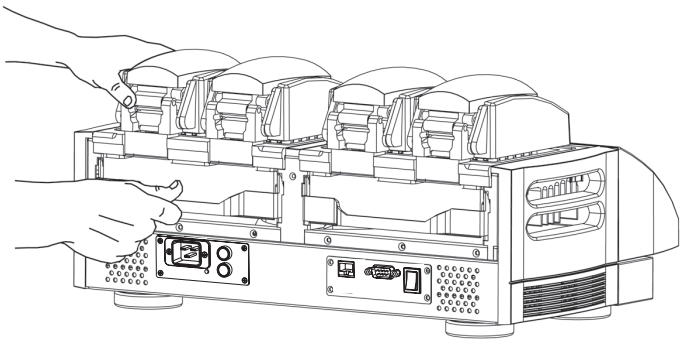
1. **Turn the DNA Engine Dyad cycler off (see the Caution above)**.
2. Pull upward on the handle. When the lock releases, you will hear a click, and the Alpha unit will slide a little toward the back of the DNA Engine Dyad cycler. The electrical connectors of the Alpha unit and the DNA Engine Dyad cycler are now disengaged, so there is little danger of electrical shock.
3. Slide the Alpha unit toward the rear of the DNA Engine Dyad cycler, about 3cm.
4. Grasp the front and back edges of the Alpha unit, and lift it out of the machine.

Figure 4-1 Installing an Alpha unit.

A



B



Opening an Alpha Unit

Grip the front edge of the top lever of the Hot Bonnet™ lid as shown in figure 4-2A, and pull upward firmly. The top lever will pop open to reveal the entire thumbwheel (see figure 4-2B). Continue pulling upward to open the lid. The Hot Bonnet lid will tip backward, revealing the entire block.

⚠ **Caution:** Do not pull on the thumbwheel to open the unit. This can damage the Hot Bonnet lid's mechanism.

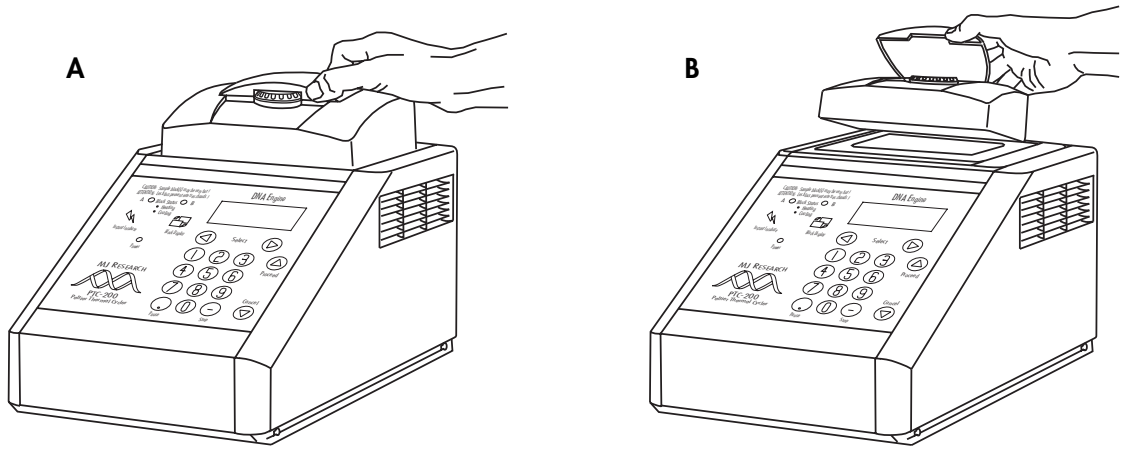
Closing an Alpha Unit

Press down on the top lever. The lever will close down over the thumbwheel as the lid closes down over the sample block. A click signifies that the Hot Bonnet lid's latch has engaged.

Selecting the Correct Sample Vessel

The DNA Engine Dyad cycler's wide variety of interchangeable Alpha units affords great scope in choosing sample vessels. Keep in mind that differences in tube and plate composition and wall thickness among the many brands available can affect reaction performance. Protocols may require some adjustment to ensure optimum performance when using a new vessel type. MJ Research offers a full range of tubes and microplates, manufactured to the specifications of each type of Alpha unit to ensure a precise fit. See Appendix 4-A of this chapter for a complete list.

Figure 4-2 Opening an Alpha unit.



0.5ml Tubes

Thick-walled 0.5ml tubes may not fit tightly in thermal cycler wells and typically provide poor thermal transfer, since these tubes were originally designed for centrifuges. For best results, we recommend using thin-walled 0.5ml tubes specifically designed for thermal cycling. The higher quality brands provide a good and consistent fit. MJ Research thin-walled 0.5ml tubes are designed for precise block fit and tight sealing of reactions down to 10µl.

Thin-Walled vs. Thick-Walled Tubes

The thickness of sample tubes directly affects the speed of sample heating and thus the amount of time required for incubations. Thick-walled tubes delay sample heating, since heat transfers more slowly through the tubes' walls. For the earliest types of thermal cyclers, this delay mattered little. These machines' ramping rates were so slow (below 1°C/sec) that there was plenty of time for heat to transfer through the tube wall to the sample, during a given incubation.

Modern thermal cyclers have much faster ramping rates (up to 2–3°C/second), so the faster heat transfer provided by thin-walled tubes allows protocols to be significantly shortened.

0.2ml Tubes

All types of thin-walled 0.2ml tubes may be used. MJ Research offers high-quality 0.2ml tubes in a number of styles, including individual and strip tubes.

Microplates

A variety of polycarbonate or polypropylene microplates can be used in Alpha units as long as they fit the wells snugly. Polypropylene microplates are usually preferred because they exhibit very low protein binding and, unlike polycarbonate microplates, do not lose water vapor through the vessel walls. This allows smaller sample volumes to be used—as little as 5–10µl.

Several varieties of microplates are available from MJ Research (see the "Tube, Microplate, and Sealing Selection Chart"), including Hard-Shell® thin-wall microplates. Hard-Shell microplates feature a skirt and deck molded from a rigid, thermostable polymer that completely resists the warping and shrinkage experienced with traditional one-component plates. The rigid skirt improves robotic handling such that stackers and robotic arms can grip and move Hard-Shell plates securely and reliably. In a separate step, thin-wall wells are molded of virgin polypropylene selected for low DNA binding and optimized for thermal cycling.

Sealing Sample Vessels

To avoid changing the concentration of reactants, steps must be taken to prevent the evaporation of water from reaction mixtures during thermal cycling. Only a layer of oil or wax will completely prevent evaporation from the surface of the reaction fluid. However, an adequate degree of protection can be achieved by sealing vessels with caps, film, adhesive seals, or mats, then cycling the samples using the heated lid to prevent condensation.

Sealing with Oil or Wax

Mineral oil, silicone oil, paraffin wax, or Chill-out™ 14 liquid wax may be used to seal samples. Use only a small amount of oil or wax; 1–3 drops (15–50µl) are usually sufficient. (Include this volume in the total volume when setting up a calculated-control protocol; see "Choosing a Temperature Control Mode" in Chapter 5.) **Use the same amount of oil or wax in all sample vessels to ensure a uniform thermal profile.**

Most paraffin waxes solidify at room temperature. The wax can then be pierced with a micropipette and the samples drawn off from below the wax. Silicone oil and mineral oil can be poured off or aspirated from tubes if the samples are first frozen (–15° to –20°C). The samples are usually pure enough for analysis without an extraction.

Chill-out liquid wax (available from MJ Research) is an easy-to-use alternative to oil. This purified paraffinic oil solidifies at 14°C and is liquid at room temperature. By programming a hold at low temperature, the wax can be solidified at the end of a run. A pipette tip can then be used to pierce the wax in the tubes and remove the samples. The wax is available in a clear, optical-assay grade or dyed red to assist in monitoring its use. The red dye has no adverse effects on fluorescent gel analysis of reaction products.

Sealing with the Hot Bonnet Lid

The Hot Bonnet's heated inner lid maintains the air in the upper part of sample vessels at a higher temperature than the reaction mixture. This prevents condensation of evaporated water vapor onto the vessel walls and lid, so that solution concentrations are unchanged by thermal cycling. The Hot Bonnet lid also exerts pressure on the tops of vessels loaded into the block, helping to maintain a vapor-tight seal and to firmly seat tubes or the plate in the block.

Caps, film, adhesive seals, or mats must be used along with the Hot Bonnet lid to prevent evaporative losses.

Note: When tubes are cooled to below-ambient temperatures, a ring of condensation may form in tubes above the liquid level but below the top of the sample block. This is not a cause for concern since it occurs only at the final cool-down step, when thermal cycling is complete.

Microseal® 'A' film offers a quick alternative to sealing microplates or arrays of tube strips. This film is specially designed to seal tightly during cycling, yet release smoothly to minimize the risk of aerosol formation and cross-contamination of samples. Microseal 'A' film is easily cut for use with fewer than 96 samples.

Microseal® 'B' adhesive seals feature an aggressive adhesive, effective from –20°C to 110°C, which allows secure sample storage or transport before and after cycling. The clear polyester backing allows easy inspection of sample wells. Microseal 'B' clear, adhesive seals are ideal for thermal cycling in all polypropylene and polystyrene microplates.

Microseal® 'M' rubber sealing mats are an economical means to seal 96-well microplates. An array of 96 dimples on the mat helps orient it on the microplate and prevents the mat from sticking to the heated lid. The mats may be cleaned with sodium hypochlorite (bleach) for reuse, and they are autoclavable.

Adjusting the Hot Bonnet Lid's Pressure

The pressure exerted by the Hot Bonnet lid must be manually adjusted to fit the sample vessels being used. Once set, the Hot Bonnet lid can be opened and closed repeatedly without readjustment as long as neither the tube or microplate type nor the sealing method is changed. Any change in vessel type or sealing method requires readjustment of the Hot Bonnet lid.

Follow these steps to adjust the pressure exerted by the inner lid:

1. Make sure the block's wells are clean. Even tiny amounts of extraneous material can decrease thermal conduction and interfere with the proper seating of a microplate or tubes.

2. Open the Hot Bonnet lid. Turn the blue thumbwheel all the way counterclockwise to completely raise the inner lid.

3. Load either a microplate or at least eight individual tubes into the sample block. The inner lid pivots around a central point, so it is important to distribute individual tubes evenly: load at least four tubes in the center of the block and at least one tube in each of the four corners of the block. If using a sealing film or mat, apply it to the loaded microplate according to the manufacturer's directions.

4. Close the Hot Bonnet lid by pressing down on the top lever. Turn the thumbwheel clockwise to lower the inner lid onto the loaded microplate/tubes. The thumbwheel turns easily at first since the inner lid has not yet come into contact with anything. Stop turning the thumbwheel when you feel increased resistance, which indicates that the inner lid has touched the microplate/tubes.

5. For microplate sealing films or mats that require additional pressure, turn the thumbwheel clockwise an extra half turn past the point of initial contact to set an appropriate lid pressure.

⚠ **Caution:** Do not turn the thumbwheel more than three-quarters of a turn. This can make it hard or impossible to close the lid and puts excessive strain on the latch holding the lid closed.

An extra half to three-quarters of a turn ensures the correct pressure for most types of reaction vessels. Some empirical testing may be required to determine the optimum pressure required for certain vessels. Once this pressure has been determined, the thumbwheel position may be marked with a colored marking pen or piece of tape.

Note: As an aid in gauging how much the thumbwheel has been turned, mark it at the quarter turn positions, or every sixth "bump" on the thumbwheel (there are 24 total "bumps").

Loading Sample Vessels into the Block

When using a small number of tubes, load at least one empty tube in each corner of the block to ensure that the Hot Bonnet lid exerts even pressure on the sample tubes (see "Adjusting the Hot Bonnet Lid's Pressure," above).

To ensure uniform heating and cooling of samples, sample vessels must be in complete contact with the block. Adequate contact is ensured by always doing the following:

- Ensure that the block is clean before loading samples (see Chapter 9 for cleaning instructions).
- Firmly press individual tubes or the microplate into the block wells.

Using Oil to Thermally Couple Sample Vessels to the Block

With two exceptions (see below), MJ Research does not recommend using oil to thermally couple sample vessels to the block, for the following reasons:

- Calculated-control protocols do not run accurately when oil is used.
- Oil traps dirt, which interferes with thermal contact between vessels and the block.

⚠ **Caution:** If you use oil in the block, use only mineral oil. **Never use silicone oil.** It can damage the Alpha unit.

One exception to this recommendation involves the use of volatile radioactive ^{35}S nucleotides. A small amount of oil in the block can help prevent escape of these compounds. See Appendix 4-B of this chapter for important information regarding safe use of these compounds in polypropylene tubes and polypropylene and polycarbonate microplates. A second exception involves the use of thick-wall 0.5ml tubes. Certain brands of these tubes fit poorly in the block, in which case, oil may somewhat improve thermal contact. Whenever possible, use high-quality thin-wall tubes intended for thermal cycling (see Appendix 4-A of this chapter for a tube and plate selection chart).

Using the Optional Probe

The probe consists of a precision thermistor mounted in a thin-walled plastic tube. A thin wire, encased in a small plastic tube, runs from the thermistor to the probe's plug, which is inserted into a slot at the back of the Alpha unit (see figure 4-3). A small amount of oil is added to the probe tube to serve as the representative sample. The tube is loaded into the block, where it can serve as the control reference for any programmed target temperature between 0˚ and 100˚C.

When a probe-control protocol is run, the DNA Engine Dyad cycler controls block temperature to keep the probe vessel at the programmed temperature, using feedback information from the thermistor. (See Chapter 5 for information on programming protocols for probe control.) **Probe control cannot be used with heated-lid protocols**.

Note: Because the thermal characteristics of a probe never precisely match those of an actual sample, calculated control is often a better choice than probe control.

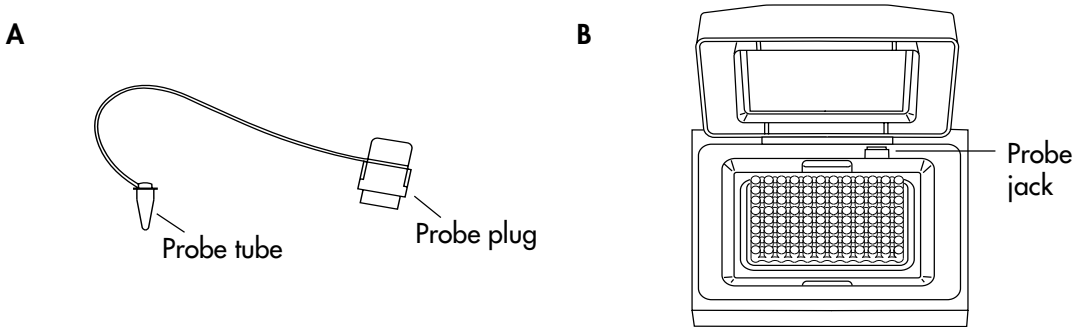
Customizing the Probe Vessel

For the most precise control of sample temperatures, install the probe's thermistor in the same type of tube that the samples will be placed in. This is particularly important when the sample tubes have much thicker walls than the probe's tube.

Follow these steps to customize the probe vessel:

1. Cut the hinge to the probe tube's lid, if there is one. Remove the lid and the attached amber-colored thermistor.
2. Remove the lid from the new probe tube. Add oil to the probe tube as described below under "Adding the Oil."

Figure 4-3 A, Probe. B, Location of probe jack.



3. Gently place the thermistor in the new tube, and snap the lid closed. Make sure that the lid from the original probe tube fits the new tube tightly. The probe wire may touch the sides of the tube. The thermistor should rest on the bottom of the tube.

⚠ **Caution:** The thermistor is extremely fragile. Handle it with great care.

Adding the Oil

Viscous oils are the best choice for the probe tube's representative sample. They closely mimic the thermal characteristics of buffer solution, which changes temperature sluggishly due to the high specific heat of water. MJ Research recommends using heavy mineral oil, for the following reasons:

- The calculations required to determine the correct volume of oil are easy.
- It is widely available and inexpensive.

Add mineral oil to the probe tube in the following proportions: 1X the volume of the buffer in an individual tube, plus 1X the volume of oil overlay if one is used. **It is important to use the correct amount of oil, so that the representative sample changes temperature at the same rate as the actual samples.** To add the oil, open the sample tube and pipette in the appropriate amount. The oil must completely cover the thermistor.

Light and heavy silicone oil may also be used but necessitate more complex calculations to determine the amount to add to the probe tube. See Appendix 4-C of this chapter for information on using these oils.

Note: Use only mineral oil or silicone oil as the representative sample. Do not use paraffin wax or Chill-out™ liquid wax, or the probe readings will not be accurate.

⚠ **Caution:** Do not use water, saline, or any other aqueous solution as a representative sample. Aqueous solutions will destroy the thermistor.

Loading and Connecting the Probe

Seat the probe tube in the center of the block (see figure 4-4). If oil is used to thermally couple samples to the block, it must also be used on the probe tube (see "Using Oil to Thermally Couple Sample Vessels to the Block").

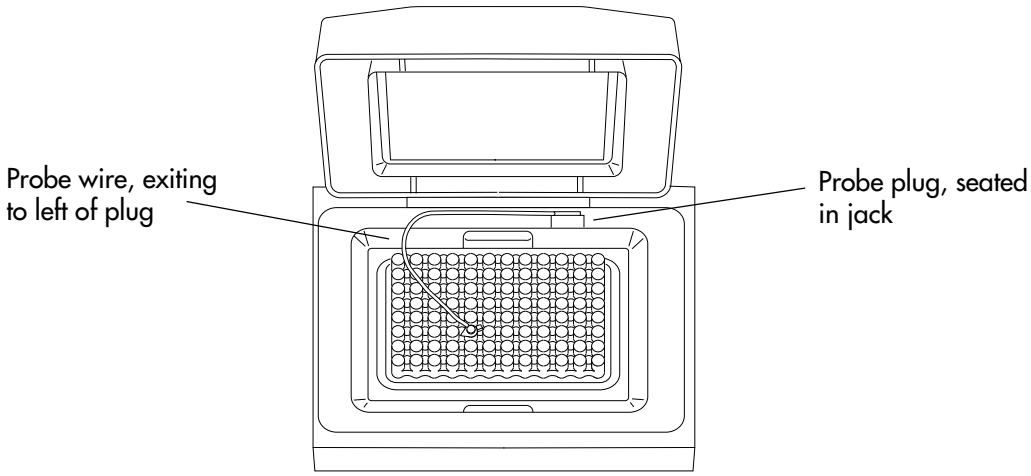
Plug the probe into the jack at the back of the block, so that the wire is to the left of the plug (see figure 4-4).

Detecting a Faulty Probe

If the DNA Engine Dyad cycler detects that the probe is broken or missing when a protocol begins running, the protocol's temperature control method is automatically switched from probe control to calculated control, and the following message is immediately displayed: "Probe Sensor Failure, Used Calc Control".

If the probe malfunctions **during** a protocol run, the temperature control method is also switched to calculated control. When the run finishes, the following message is displayed: "Calc control, Probe not present".

Figure 4-4 Correctly inserted probe.



Appendix 4-A Tube, Microplate, and Sealing System Selection Chart

The following sample vessels and sealing options are recommended for use with the DNA Engine Dyad cycler and are available from MJ Research. To place an order, call 888-729-2165 or fax 888-729-2166.

Key

✔ Reaction vessel fits block/sealing option fits reaction vessel without modification.

✂ Reaction vessel/sealing option can be cut to fit.

MJ Research Thermal Cycler Blocks					Reaction Vessels		Sealing Options for Oil-Free Cycling						
384	96 (0.2ml)	48 (0.2ml)	60 (0.5ml)	30 (0.5ml)	Description	MJ Research Catalog #	Microseal 'A' film MSA-5001	Microseal 'B' seals MSB-1001	Microseal 'M' mat MSM-1001	Microseal 'P' pad MSP-1001	8-Strip caps TCS-0801	12-Strip caps TCS-1201	Chill-out wax CHO-series
✔					Microseal™ skirted 384-well microplates	MSP-series	✔	✔		✔			✔
	✔				Microseal™ skirted 96-well microplates	MSP-series	✔	✔	✔		✔	✔	✔
	✔				Hard-Shell™ skirted 96-well microplates	HSP-series	✔	✔	✔	✔	✔	✔	✔
	✔	✂			Multiplate™ unskirted 96-well microplates	MLP-series MLL-series	✔	✔	✔	✔	✔	✔	✔
	✔	✔			Multiplate™ unskirted 48-well microplates	MLP-series	✔	✔	✔	✔	✔	✂	✔
	✔	✔			Multiplate™ unskirted 24-well microplates	MLP-2401	✔	✔	✔	✔	✔	✂	✔
	✔	✔			Multiplate™ unskirted 25-well microplates	MLP-2501	✔	✔	✔	✔	✂	✂	✔
	✔				"Concord" skirted 96-well microplates	CON-9601	✔						✔
	✔	✔			8-strip 0.2-ml tubes	TBS-series TLS-series	✔		✔	✔	✔	✂	✔
	✔	✂			12-strip 0.2-ml tubes	TBS-series	✔		✔	✔	✂	✔	✔
	✔	✔			0.2-ml tubes, no caps	TBI-series	✔		✔	✔	✔	✔	✔
	✔	✔			0.2-ml tubes w/caps	TFI-0201 TWI-0201							✔
			✔	✔	0.5-ml tubes w/caps, thin wall	TBI-series							✔

Note: "Concord" microplates are made from polycarbonate plastic, which is more prone to poor sealing and vapor leakage during stringent thermal cycling.

Appendix 4-B Safety Warning Regarding Use Of ^{35}S Nucleotides

Some researchers have experienced a problem with **radioactive contamination** when using ^{35}S in thermal cyclers. This problem has occurred with all types of reaction vessels.

The Problem

When ^{35}S nucleotides are thermally cycled, a volatile chemical breakdown product forms, probably SO_2. This product can escape the vessel and contaminate the sample block of a thermal cycler, and possibly, the air in the laboratory. Contamination has been reported with microassay plates, 0.2ml tubes, and 0.5ml tubes.

96-Well Polycarbonate Microplates

These microplates present the largest risk of contamination. Polycarbonate is somewhat permeable both to water and the ^{35}S breakdown product. This problem is exacerbated when polycarbonate plates are held at high temperatures for long periods of time, or when the plates are sealed for oil-free thermal cycling.

0.2ml Polypropylene Tubes and Polypropylene Microplates

These tubes are manufactured with very thin walls to enhance thermal transfer. The thin walls are somewhat fragile and can "craze" or develop small cracks when subject to mechanical stress. Undamaged thin polypropylene tubes may also be somewhat permeable to the ^{35}S breakdown product. Either way, there have been reports of ^{35}S passing through the walls of 0.2ml tubes of several different brands during thermal cycling. No data are yet available on radioactive contamination with polypropylene microplates.

0.5ml Polypropylene Tubes

Contamination problems are rarer with this type of tube, but instances have been reported.

The Solution

1. Substitute the low-energy beta emitter ^{33}P in cycle sequencing. ^{33}P nucleotides are not subject to the same kind of chemical breakdown as ^{35}S nucleotides, and they have not been associated with volatile breakdown products.

2. If ^{35}S must be used, three things will help control contamination: an oil overlay inside the tubes, mineral oil in the thermal cycler outside the tubes, and use of thick-walled 0.5ml tubes. Always run ^{35}S thermal cycling reactions in a fume hood, and be aware that vessels may be contaminated on the outside after thermal cycling. Please be certain that you are using the appropriate detection methods and cleaning procedures for this isotope. Consult your radiation safety officer for his or her recommendations.

   If mild cleaning agents do not remove radioactivity, harsher cleaners may be used occasionally and carefully. Users have suggested the detergent PCC-54 (Pierce Chemical Co., Rockford, Illinois; Pierce Eurochemie B.V., Holland), Micro Cleaning Solution (Cole-Parmer, Niles, Illinois), and Dow Bathroom Cleaner (available in supermarkets).

⚠ **Caution:** Harsh cleaning agents (such as those above) are corrosive and must be *thoroughly* rinsed away within a few minutes of application. They can eat away the surface finish of the blocks.

Appendix 4-C Using Silicone Oil in the Probe Tube

The following light and heavy silicone oils may be used instead of mineral oil as the representative sample in a probe tube:

- **Dow Corning #200 light silicone oil** (dimethypolysiloxane, Sigma #DMPS-5X)

  Density: 0.97g/ml
  Viscosity: 50cs

  Volume to use: 1.7 x volume of buffer in individual sample tube, plus one volume of oil overlay.

- **Dow Corning #200 heavy silicone oil** (dimethypolysiloxane, Sigma #DMPS-V)

  Density: 0.97g/ml
  Viscosity: 5cs

  Volume to use: 2.7 x volume of buffer in individual sample tube, plus one volume of oil overlay.

Note: Use these oils only in the proportions outlined above. Using them in any other proportion (for example, 1:1 with sample tube volumes) will lead to inaccurate sample heating.

5 Creating Programs

In this chapter, we will revisit the setup of the front panel, specifically those items used in program input. We will describe the conventions used, as well as the various programming steps and what they accomplish. We make suggestions regarding the translation of a cycle sequencing protocol into a Dyad program. Finally, we will use a cycle sequencing example to illustrate the programming process, step by step.

Front Panel Setup

The various components of the Dyad control panel (see figure 2-2) enable the operator to enter, navigate, and manipulate programs. These programs are necessary to control the various dynamic capabilities of the Dyad cycler.

Note: Chapter 4 covers the basic operation of the DNA Engine Dyad cycler. Please read Chapter 4 for a complete description of the control panel and power-up procedures.

Let's review. The control panel components include:

Display Screen

This is a 1/4 VGA display screen, approximately 10cm x 12.5cm, located at the left side of the control panel. It displays all Dyad cycler operating parameters, and can be controlled by the cursor buttons, touch pad or external mouse, and the numeric keypad.

⚠ **Caution:** Unlike the touch pad, the display screen is not a touch screen and should not be used to enter programming items. Please avoid touching the display screen.

Cursor Keys

These are four cursor keys located to the right of the display screen. They can be used to navigate through various menu and selection items.

The use of these keys is optional as ALL screen selections can be done using the touch pad or the external mouse device.

The **up/down** keys are primarily used to scroll vertically through various submenus, and to toggle through selection options in a list. The **left/right** keys are used to navigate through menu bars, and to move through all available buttons or options in any given window.

Numeric Keypad

This is located to the right of the cursor buttons and consists of a typical numeric keypad (numbers 0 through 9) and <<ENTER>> key. There is also a backspace/delete key and a decimal button. The numeric keypad is used to enter parameters such as temperature, hold time, and cycle iterations.

Touch Pad

Located on the right side of the control panel, and bearing the MJR logo, the touch pad is a touch-sensitive mouse emulation device, approximately 8cm x 6.4cm. Essentially, all program maneuvers and navigation can be accomplished using the touch pad and the numeric keypad. The touch pad is used to move a pointer that is visible on the display screen.

Fingertip movement on the touch pad will result in similar pointer movement. Hence, a fingertip, dragged from left to right on the touch pad, will result in movement of the pointer from left to right on the display screen.

Once the pointer is positioned over a menu or selection item on the display screen, a single tap of the touch pad with a fingertip will implement the command. The only exception is during the editing of programming steps. Selection of programming steps for editing requires a double tap. Tapping the touch pad has the same effect as the left touch pad button.

✔ **Tip:** When programming, we recommend not to rest your finger on the touch pad as you may unintentionally select a field on the display.

Note: If you have chosen to enable the external mouse device (see the "Setting Up the DNA Engine Dyad Cycler" section in Chapter 3), the touch pad can not be used to input programming commands. The cycler will recognize either the touch pad OR a mouse, but not both input devices simultaneously.

Touch Pad Buttons

These two buttons are located below the touch pad. Once the pointer is positioned via the touch pad over a menu or selection item on the display screen, the left button can be used to implement commands. Pressing this button has the same effect as a single tap on the touch pad.

Note: The right touch pad button is reserved for future programming functions.

Programming Conventions

Before starting the Dyad programming process, let's review some "common ground" conventions used here.

- << >> will be used to indicate actual keys on the control panel, such as <<ENTER>>, <<1>> and <<LEFT>>.
- < > will be used to indicate windowed menu items or buttons, such as <PROGRAMS>, <RUN> and <TOOLS>.
- *Italics* will be used to indicate windowed items that are not drop down menu items or buttons, such as *Calculated*, *Block*, and *Tracking*. Typically, these will be parameter selection items.
- ***Select*** is meant to be synonymous with ***click on***, ***point-and-click***, and any phraseology implying selection of menu or option items with a mouse. Particularly with the Dyad cycler, ***select*** should symbolize any physical selection on the Dyad input devices (touch pad/mouse, numeric keypad, cursor keys). This includes single or double taps on the touch pad, pressing of the left touch pad button, or pressing the <<Enter>> button on the numeric keypad.

The Elements of a Program

Dyad programs consist of a combination or series of steps and setup parameters that represent protocol requirements.

Note: The procedures involved in actually entering these steps will be described in subsequent pages, but please familiarize yourself with the types of steps used to create Dyad programs.

The considerations behind choosing various elements will be explained further in the "Considerations During Program Creation" section. The following is a summary of the individual program elements and their basic functions.

Temperature Control Mode: This parameter defines the temperature control algorithm used during the program run. The three different modes include ***Calculated***, ***Block*** and ***In-Sample Probe***. Due to the expected lag of sample temperature behind block temperature, the Dyad cycler can use calculated mode to compensate accordingly. The Dyad cycler defaults to ***Calculated***. Refer to the "Choosing a Temperature Control Mode" section below for additional information.

Lid Control Mode: The Hot Bonnet™ heated lid can be programmed to minimize condensation by keeping the upper surface of the reaction vessel at a temperature slightly greater than that of the sample itself. The three available lid modes include ***Off***, ***Tracking***, and ***Constant***. The Dyad cycler defaults to ***Constant***. Refer to the "Choosing a Lid Control Mode" section below for additional information.

Temperature step: This sets incubation temperature and duration. The Dyad cycler ramps the sample to this temperature at its maximum rate unless ramp modifying instructions are added to the program (advanced mode only). The maximum rate of heating is 3°C/sec and cooling is 2°C/sec for all standard Alpha units (maximum rate of heating is 1.2°C/sec for the Twin Towers Alpha unit).

Gradient step: This establishes a temperature gradient across a 96-well sample block. The range of any single gradient can be as great as 24°C or as small as 1°C from left to right across the block. The maximum programmable temperature is 105°C; the minimum programmable temperature is 30°C.

GoTo step: Directs the program to cycle back to an earlier step a specified number of times.

Lid step: Directs a Power Bonnet™ motorized lid to automatically open or close (only available in advanced programs).

End step: Automatically included, this instructs the Dyad cycler to shut down its heat pump because the program is complete.

These additional program modifications are available in advanced programs (see the "Types of Programs" section immediately following for more information on advanced programs):

1. **Increment Temp:** Modifies a temperature step to allow a "per cycle" increase or decrease of temperature (0.1°C to 10.0°C per cycle) each time the step is executed. This feature is useful when annealing stringency is a consideration such as in a touchdown program.

   In a touchdown program, the annealing temperature begins higher than the calculated temperature, and incrementally decreases each cycle, first reaching, and eventually falling below the calculated annealing temperature. With the reaction beginning at a temperature favoring high stringency in hybridization and incrementing to lower stringency, the reaction favors the desired product by creating a high proportion of signal relative to noise in the early amplification cycles.

2. **Extend Time:** Modifies a temperature step to allow a "per cycle" lengthening or shortening of a temperature step hold (by 1–60 sec/cycle) each time a step is executed. This capability is useful for slowly increasing (typically by 2 to 5 seconds per cycle) the hold time during an extension step. The number of bases that a polymerase must synthesize during the extension step increases in later cycles because there are more template molecules, because there are fewer active polymerase molecules, or both. The extra time can allow synthesis to be completed.

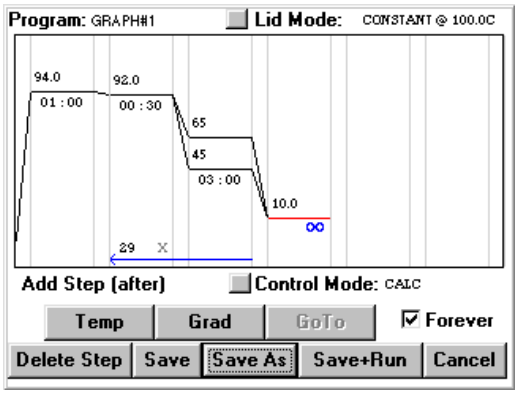
3. **Slow Ramp:** Temperature modification which allows for slower temperature ramping than the default maximum rate of 3.0°C/sec. The minimum rate currently allowed is 0.1°C/sec. Slower ramp times than this may be achieved using a combination of increment and goto steps. Contact MJR Technical Support at 888-652-9253 for details.

4. **Beep:** Modifies a temperature step or ramp step so the instrument will beep when the target temperature is reached.

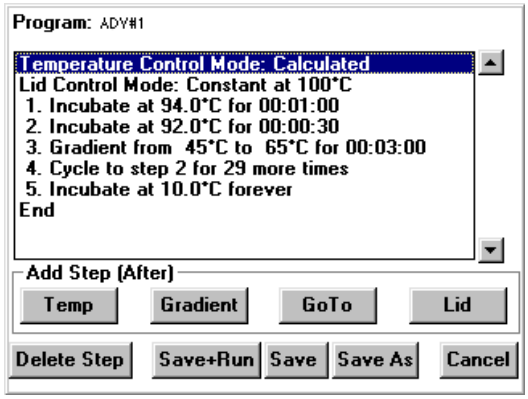
Types of Programs

There are two types of Dyad programs, graphical and advanced. Graphical programming features a graphical interface and a graphical representation of the program steps. Advanced programming features a text-based interface and a descriptive listing of the program steps.

Graphical program



Advanced program



Graphical Programs

Creating a graphical program is desirable if you prefer the graphical programming interface and/or wish to quickly enter a program that:

- Does not contain more than a total of six temperature and/or gradient steps
- Does not contain temperature or gradient steps that contain modifications (i.e., increment temp, extend time, slow ramp, beep)
- Does not contain more than one goto step
- Does not contain a temperature incubation below 0°C
- Does not contain a step with an incubation lasting more than 1 hour 39 minutes and 59 seconds (99:59). Forever incubations excepted.

In addition to the speed with which they can be entered, another benefit of graphical programs is that they can be quickly edited. This can be particularly useful if you tend to repeatedly run the same general protocol with limited changes (e.g., varying annealing temperatures).

To create a graphical program, refer to the "Entering a Program Using Graphical Mode" section below.

Advanced Programs

Advanced programs offer all of the Dyad programming features with the exception of the graphical programming interface. Creating an advanced program is desirable if you wish to enter a program that:

- Contains many steps
- Contains temperature or gradient steps with modifying instructions (i.e., increment temp, extend time, slow ramp, beep)
- Contains multiple goto steps
- Contains a temperature incubation in the range of –1°C to –5°C
- Contains an incubation lasting between 1 hour 40 minutes and 18 hours (incubation times shorter than 1 hour 40 minutes and forever incubations are also allowed.)

While all graphical programs can be opened and edited in advanced mode, only a subset of advanced programs can be opened and edited in basic mode. Advanced programs that meet the criteria outlined above for graphical programs can be opened in basic mode (see "Opening a Program" in Chapter 6 for more information).

To create an advanced program, refer to the "Entering a Program Using Advanced Mode" section below.

Designing a New Program

The first step in designing any program is the translation of your experimental protocol into Dyad program steps. We suggest writing all steps until you are reasonably comfortable with Dyad programming.

For purposes of this explanation, we will be working with a cycle sequencing example. First, we will write down the raw steps, then make some modifications with the parameters that were described in the previous section, "The Elements of a Program", and then determine what our final program should be. The actual implementation and entering of program steps will be covered in a later section.

Note: You will soon become familiar with Dyad program design and be able to enter steps directly from experimental protocols. However, we strongly suggest following these steps the first few times through, as they will probably save troubleshooting time later.

Let's Start with an Example

Assume you have the necessary components for a 30-cycle sequencing reaction, and you have calculated the annealing temperature of your oligonucleotide to be 60°C. Please note that MJ Research recommends using 92°C as the default denaturation temperature during cycling steps. The resulting raw program you write may look something like this:

Raw program:

1. 92°C for 30 seconds
2. 60°C for 3 minutes
3. 92°C for 30 seconds
4. 60°C for 3 minutes
5. 92°C for 30 seconds
6. 60°C for 3 minutes

[continues for a total of 60 lines]

The Goto Option

At 60 lines, our program is large, unwieldy and would take time to input. At step 3, repetition can be reduced with the addition of a goto statement:

Raw program:

1. 92°C for 30 seconds
2. 60°C for 3 minutes
3. Goto step 1, 29 more times
4. END

One of the most important factors in the program writing process is identifying repetitive steps. These can then be enclosed in a goto loop as shown above.

Considerations During Program Creation

Once you have written the body of your raw program, there are decisions to make before creating your Dyad program. They concern how your steps should be implemented. These decisions involve the following:

- Temperature control mode
- Lid control mode
- Temperature ramping rate (advanced mode only)
- Temperature hold time
- Temperature time extend (advanced mode only)
- Temperature increment (advanced mode only)
- Temperature gradient (available with the 96-well Alpha unit only).
- Outside of the example protocol: other considerations

Choosing a Temperature Control Mode

The Dyad cycler can control incubation temperature in three possible ways, each of which has different implications for the speed and accuracy of sample heating. These include ***Calculated Control***, ***Block Control***, and ***In-Sample Probe Control***.

Calculated Control

When using calculated control, the Dyad cycler estimates sample temperatures based on the block's thermal profile, the rate of heat transfer through the sample tube or slide, and the sample volume or mass. Since this estimate is based on known quantities and the laws of thermodynamics, sample temperatures are controlled much more accurately than with block or probe control.

Since the sample temperature will always lag behind the block temperature, the Dyad cycler can adjust the block temperature to bring samples of a specific volume in a specific vessel type to programmed temperatures. This is done through optimized "overshoots" of the block temperature by a few degrees for a few seconds, which bring samples to the desired temperature more quickly.

Calculated control is also the method of choice for most types of programs because it yields the most consistency, reliability, and speed. Calculated control provides for shorter protocols in three ways:

1. Brief and precise block temperature overshoots are used to bring samples to desired incubation temperature rapidly.

2. Incubation periods are timed according to how long the samples, not the blocks, reside at the target temperature.

3. The instrument automatically compensates for vessel type and reaction volume.

Note: We will choose calculated control for our example.

Block Control

The Dyad cycler maintains the block at the programmed temperature, independent of sample temperature. This mode of temperature control is common to older models of thermal cyclers.

Block control provides less accurate control of sample temperatures than calculated control provides. Under block control, the temperature of samples will lag behind the temperature of the block. The length of the time lag depends on the vessel type and sample volume but typically is between 10 and 30 seconds. Block control is chiefly used to run protocols developed for other thermal cyclers that use block control, or if you use the <Instant> command to incubate samples at a set temperature for long periods of time.

In-Sample Probe Control

The Dyad cycler adjusts the block's temperature to maintain the probe at programmed temperatures. You may purchase an in-sample probe from MJ Research, Inc. The in-sample probe is comprised of a temperature probe placed inside a typical capped tube. A thin cable exits the top and may be plugged into the Alpha unit.

Probe control is available for unusual circumstances that may require it. Ordinarily, though, it should be used with caution. While the Dyad cycler will have no trouble heating the probe to the target temperature, if the probe is seated or prepared differently from the sample tubes, actual sample temperatures can vary widely from the probe's temperature. Probe control cannot be used with microplates or slides, or in conjunction with the heated lid.

Modifying Block- and Probe- Control Programs for Calculated Control

Probe-control programs will generally run well under calculated control, with no modification other than changing the method of temperature control. Block-control programs can be changed to calculated control by subtracting at least 15–20 seconds from each temperature step. Some empirical testing may be required to adjust modified programs for optimum performance. We generally recommend not reducing the incubation time for a step below 5 seconds while in calculated control mode.

Modifying a Program Designed for a Different Machine

The ramp programming step can be used to adapt programs designed for thermal cyclers with slower maximum heating and cooling rates than the Dyad cycler. In addition, a given protocol will occasionally work better with a slower rate of temperature change; the ramp step can be used to optimize the program for such a protocol.

Choosing a Lid Control Mode

When a sample is heated, condensation on the tube cap or plate cover can take place. This changes the volume of the sample, the concentration of components, and the kinetics of the enzymatic reaction. The Hot Bonnet™ heated lid minimizes condensation by heating the upper surface of the reaction vessel to a temperature slightly greater than that of the sample itself. The Dyad cycler can control lid temperature in three possible ways: ***Constant***, ***Tracking***, or ***Off***.

Constant Mode: This mode maintains the inner lid surface at a specific temperature regardless of sample temperature. When using constant mode, specify a lid temperature at least 5°C higher than any temperature used in the protocol.

Note: We will choose to maintain a constant lid temperature of 100°C in our example program.

Tracking Mode: Offsets the temperature of the heated inner lid a minimum specified number of degrees Celsius in comparison to the temperature of the sample block. Tracking is useful for protocols with long incubations in the range of 30-70°C, where it may be undesirable to keep the lid at a very high temperature. An offset of 5°C above block temperature is adequate for most protocols.

Off: No power is applied to the heated lid. In this mode, condensation will occur at a rate consistent with the incubation temperature and the type of tube or plate sealant being used. This option is recommended only when using an oil or wax overlay.

Choosing a Temperature Ramping Rate (Advanced Mode Only)

Fast thermal ramping between incubation steps can often help reduce overall reaction times by 10% to 30% and may help reduce production of non-specific products. The Alpha™ units use multiple zones of thermal control, which allow rapid ramp rates to be balanced with temperature uniformity.

The Dyad cycler is capable of ramping temperatures in a range of –5.0°C to 105.0°C, but no more than 30°C below ambient temperature. The ramp rate can be as low as 0.1°C/sec, or as fast as 3.0°C/sec. Slower ramp times may be achieved using a combination of increment and goto steps. Contact MJR Technical Support at 888-652-9253 for details. If a ramp rate is not programmed, the default will be at maximum.

Choosing a Temperature Hold Time

Because of the calculated melting temperature (Tm) of a DNA hybrid, DNA polymerase processivity, and reaction kinetics, it may be possible to generalize conditions regarding thermal-cycling protocols. However, decisions on denaturation, annealing or extension hold times will be reaction specific and should be optimized.

A target temperature can be held for as little as 1 second, or up to forever, should a protocol require an extended incubation period. In graphical programs, the maximum programmable hold time for a step is 1 hour 39 minutes and 59 seconds (99:59), with the exception of a forever incubation. In advanced programs, the maximum programmable hold time is 18 hours, with the exception of a forever incubation.

Choosing A Thermal Gradient

Molecular biology labs routinely optimize annealing and denaturing temperatures for thermal cycling reactions. Optimization is critical, but not always easy. The Tm ('melting temperature') of an oligonucleotide can be estimated using an empirically derived correlation which considers a combination of DNA length, G+C content, and salt concentration. However, since the Tm is only an estimate, the "true" annealing temperature may need adjusting in the actual experiment. This optimization involves repeating a reaction at several different annealing temperatures, which requires a great deal of time and monopolizes the instrument while several experiments are run in tandem. To complicate matters further, similar time-consuming experiments may also be required for denaturing temperature optimization.

The Dyad cycler programmable temperature gradient feature allows for optimization of an incubation temperature in a single experiment by analyzing a number of different temperatures simultaneously. The thermal gradient delivers a controlled thermal difference, left to right, across the sample block. This will result in a precisely defined temperature gradient that is repeatable from experiment to experiment. The range of temperatures that can be achieved from left to right across a 96-well Alpha unit can be as small as 1°C or as great as 24°C. The maximum programmable temperature is 105°C; the minimum programmable temperature is 30°C.

Note: The programmable temperature gradient feature is only accessible if a 96-well Alpha™ unit(s) is mounted in the Dyad cycler. The gradient feature is not compatible with other types of Alpha units.

The temperature of any well or column in the sample block may be displayed using the <Gradient Calculator> available from the <Tools> drop-down menu in the Status window.

Since our oligonucleotide annealing temperature is not optimized, we will replace our annealing step with a gradient step. We will optimize in the range of 45°C to 65°C.

Your written program should now appear as follows:

Raw program:

Use calculated temperature control mode

Use constant lid control mode at 100°C

1. 92°C for 30 seconds
2. Gradient from 45°C to 65°C for 3 minutes
3 Goto step 1, 29 more times
4. END

Beyond the Example Protocol: Other Considerations

In addition to the above considerations, you can also include other protocol variations which will further optimize the yield and quality of your product.

For example, an initial extended denaturation step can serve to destroy any heat-labile nucleases and other potentially interfering components, while ensuring that the nucleic acid has been completely denatured and prepped for annealing.

In some protocols, after the final elongation step, a slow temperature ramp can also be included to ensure proper product annealing.

In addition, some protocols can include a sustained incubation at sub-ambient temperatures to preserve the integrity of the products.

We will choose an initial incubation at 94°C for 1 minute before cycling, and a final incubation of the sample at 10°C forever. Your written program might now appear as follows:

Raw program:

Use calculated temperature control mode

Use constant lid control mode at 100°C

An initial incubation at 94°C for 1 minute

1. 92°C for 30 seconds
2. Gradient from 45°C to 65°C for 3 minutes
3. Goto step 1, 29 more times
4. An incubation at 10°C forever
5. END

Now that we've made some important decisions regarding the implementation of our program, we are ready to begin entering steps.

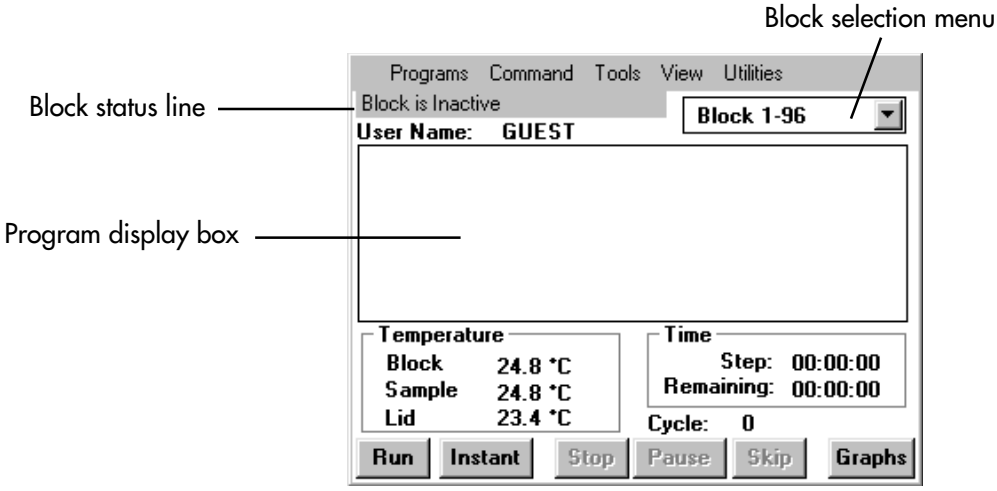
Entering Program Steps

From our example, we are ready to enter a new program. When executing a selected command via the touch pad, we will use the method of tapping the pad with a fingertip. This can also be accomplished with the left button below the touch pad, as well as the <<ENTER>> button on the numeric keypad. If the external mouse device has been enabled, this corresponds to left-clicking the mouse.

Start-up procedures for the Dyad cycler are covered in detail in Chapter 4, including start-up screens. Please review Chapter 4 before proceeding with the entering of program steps.

The Status Window

Once the Dyad cycler has completed its boot-up sequence, the Status window will be visible.



Block, sample, and lid temperatures are displayed for the convenience of the operator along with the current cycle number. The time remaining in the current step and in the program are also indicated.

At the screen bottom, the program control buttons <Run>, <Instant>, <Stop>, <Pause>, <Skip>, and <Graphs> allow the operator global or line-by-line control of the program currently loaded into memory. These will be covered in more detail in Chapter 7. The <Graphs> button can be used to display a window that simultaneously and graphically shows sample, block and lid temperatures for both Alpha units.

Note: In the Graphs window, in the same position, there is a <Status> button. By leaving your cursor in the same position in the window, and tapping the touch pad, you can toggle between the Status and Graphs windows rapidly.

The *Block Selection* menu and *Block Status* line give information about the block currently selected and its run status. The *User Name* line indicates if a particular user has been selected.

The *Program Display* box will list steps for the program currently running on the selected block.

The menu bar at the top of the Status window includes five submenus: <Programs>, <Command>, <Tools>, <View>, and <Utilities>. These submenus provide the operator with paths for maneuvering through the various Dyad software windows. For the purposes of this chapter, we will be primarily concerned with the <Programs> submenu. The other submenus will be covered in Chapters 7 and 8.

Entering a Program Using Graphical Mode

After creation of the initial program, entering a program in graphical mode essentially involves editing the graphically displayed TEMPLATE program. The TEMPLATE program will be the last, graphical program that was saved. In this section, we will address both creating an initial graphical program and editing a preexisting template.

- **Select <Programs>.**

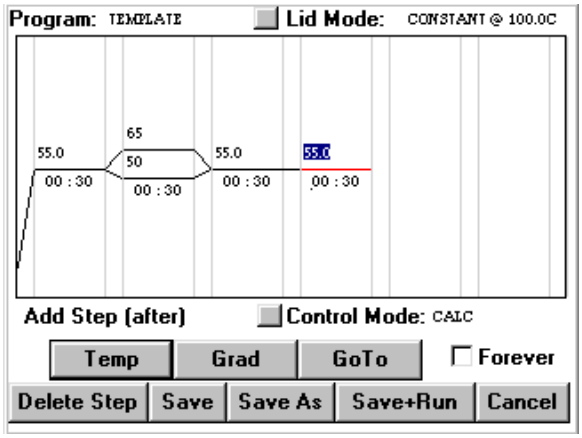
  Note: As described earlier, this involves positioning the screen cursor over <Programs> with a fingertip on the touch pad and tapping the touch pad once.

  Drop-down submenus appear, including <Open>, <New>, <Copy>, <Move>, <Delete>, <Delete Folder>, and <New Folder>.

- **Select <New>.**

  An additional menu appears allowing you to choose <Advanced Mode> or <Basic Mode>.

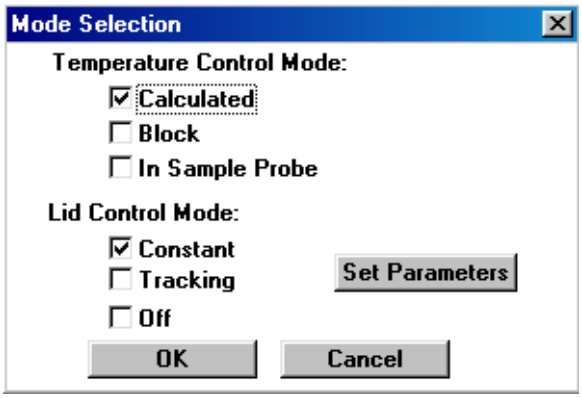
- **Select <Basic Mode>.**



The graphical programming window appears displaying no program steps or the last, saved program. In either case, the new program bears the default name, *TEMPLATE*.

Begin by choosing the temperature control mode and lid control mode for the program. Refer to the "Considerations During Program Creation" section earlier in this chapter for information on temperature and lid control modes. The current mode of temperature control is listed in the *Control Mode* field. The current mode of lid control is listed in the *Lid Mode* field. To change the control or lid mode, select the box in front of that field. In either case, the Mode Selection window appears.

Using the Mode Selection Window



For the purposes of this example, we have decided to use *Calculated* for our Temperature Control Mode.

- **Select *Calculated*.**

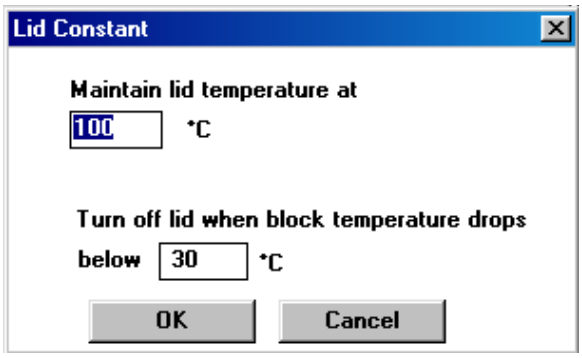
  We have decided to use *Constant* for our Lid Control Mode.

- **Select *Constant*.**

  Constant mode will allow the operator to set the parameters for the heated-lid temperature as well as the temperature at which the lid will turn off.

- **Select <Set Parameters>.**

  The Lid Constant window will appear.



Place the cursor in the *Maintain lid temperature at* field and select the field. We have decided to set the lid to a constant temperature of 100°C.

- **Enter 100 from the numeric keypad.**

  Place the cursor in the *below* field and select the field. We have decided to turn the lid off when the block drops below 30°C.

- **Enter 30 from the numeric keypad.**

- **Select <OK>.**

  We have returned to the Mode Selection window.

- **Select <OK>.**

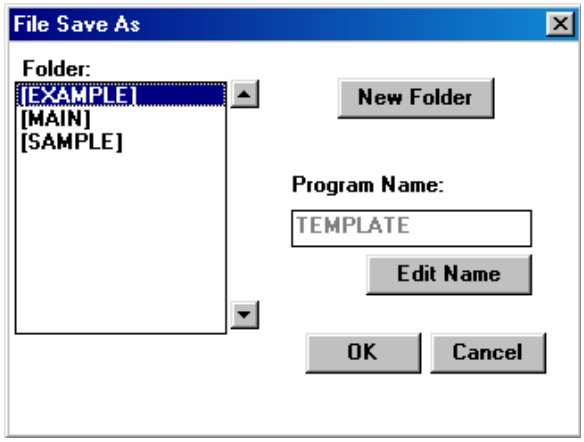
  We have returned to the graphical programming window. It is from this location that you will add steps using the <Temp>, <Gradient>, and <Goto> options. Additionally, buttons running across the window bottom provide options to <Delete Step>, <Save + Run>, <Save>, or <Save As> programs, and <Cancel> the current programming session.

Using the File Save As Window

The <Save> and <Save As> buttons are probably the most important buttons in the graphical programming window, since a program that is saved can be used or edited at a later date.

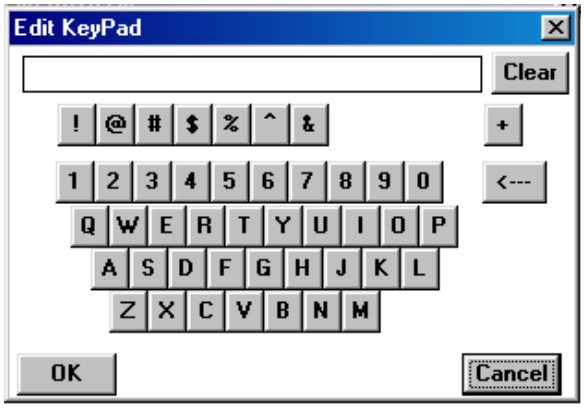
Please note that a new graphical program must be renamed using the <Save As> feature prior to initiating a run. "TEMPLATE" is not a valid name nor can a graphical program with the default name, "TEMPLATE", be run. This restriction is designed to ensure that programs are appropriately saved.

- **Select <Save As>.**



The File Save As window presents the operator with a space for entering the program name. The program will be added to the folder indicated in the *Folder* field. The <New Folder> button creates a new folder in which to store your new program.

- **Select <New Folder>.**



In this Edit KeyPad window, you can select letters that will compose the name of your new folder. Folder names cannot be longer than eight characters.

The virtual keyboard will be presented in situations where a combination of letters and numbers should be entered.

- **Select the characters "F-O-L-D-E-R-1" in succession.**

  The backspace key can be used to correct any mistakes.

- **Select <OK>.**

You will be returned to the File Save As window. The display will have changed slightly, with our newly created folder appearing in the *Folder* list. We will want to save our program in this newly created folder.

- **Position the cursor over the folder FOLDER1 and select.**
- **Select <Edit Name>.**

  Again, you are presented with the Edit KeyPad window.

- **Select the characters "G-R-A-P-H-#-1" in succession.**
- **Select <OK>.**

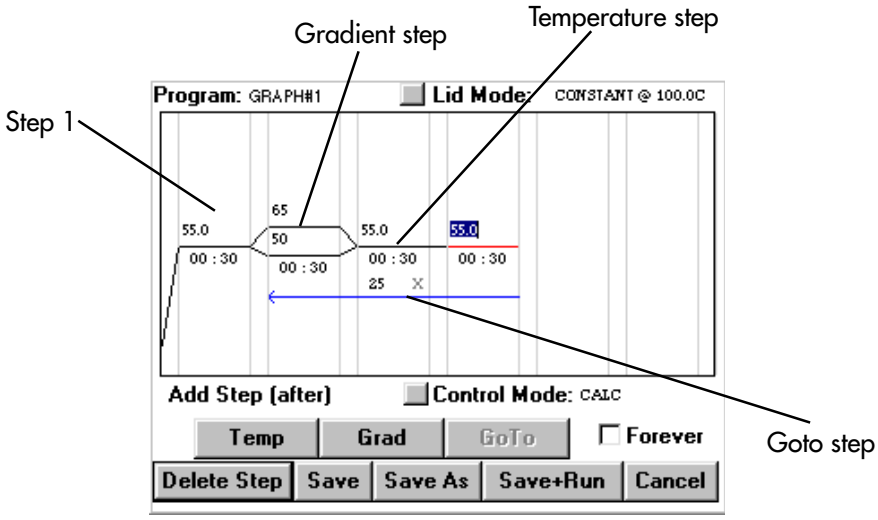
  You will be returned to the File Save As window. At this point, selecting <OK> will write your program to the hard drive. Selecting <Cancel> will bring up the programming window without saving your program.

- **Select <OK>.**

  You have returned to the graphical programming window. The new program name, *GRAPH#1*, now appears in the *Program* field.

The Graphical Programming Window

Before we begin entering program steps, let's explore the graphical programming window. The graphical programming window displays the steps of graphical programs in an arrangement of six wide columns separated by seven narrow columns. The narrow columns depict the transition phases between steps, while the wide columns depict the temperature and/or gradient steps included in the protocol. A single black line in a wide column indicates a temperature step, two black lines represent a gradient step. A goto step is indicated by a blue arrow extending from the end of the last step to be included in the loop to the beginning of the first step in the loop. The incubation temperature of the step is indicated above the step line, and the duration of the step is indicated below the step line.



Selecting a Step

When a step is selected, the line(s) or arrow depicting that step will turn red. There are several ways to select a step. You can select a step by positioning the cursor in the step's column and tapping or clicking once. A flashing insertion point will appear in the temperature field of that step. To select a specific temperature or time field (or a goto step), position the cursor, its shape will change to a text pointer, in the desired field and tap/click once to display an insertion point, or tap/click twice to highlight the entire field. Alternatively, use the left/right cursor keys to sequentially select the temperature, time, and/or number of cycles fields for the program steps.

Editing Step Parameters

If you have selected a step such that a flashing insertion point appears in the field you wish to edit, use the backspace and number keys on the numeric keypad to first delete the current value, then enter the desired temperature, time, or number of cycles. Tap the touch pad or click the mouse once to accept the change, or press the left/right cursor key once to accept the change and move to the next field. If you have selected a step such that the field you wish to edit is highlighted, use the number keys to enter the desired temperature, time, or number of cycles. Tap the touch pad or click the mouse once to accept the changes.

If an inappropriate value is entered, such as an incubation temperature of 110°C, the change will be rejected, and the default value or last valid value will reappear.

Deleting a Step

To delete a step, first select the step as indicated above such that the line or arrow depicting that step turns red. Then, select the <Delete Step> button. The selected step will be deleted and the following step will automatically be promoted.

Adding a Step

In graphical programs, a step is added directly after the step that is currently selected. Graphical programs can contain a total of six temperature and gradient steps and one goto step. To add a step, select the step that will immediately proceed the new step. Then, select either the <Temp>, <Grad>, or <GoTo> button to add either a temperature, gradient or a goto step to the program. A goto step can not be the first or only step in a program. See the sections immediately following for complete instructions on adding specific types of steps and entering step parameters.

Now, let's begin entering the steps for our example program.

Entering a Temperature Step

Recall again our raw program:

Use calculated temperature control mode

Use constant lid control mode at 100°C

An initial incubation at 94°C for 1 minute

1. 92°C for 30 seconds
2. Gradient from 45°C to 65°C for 3 minutes
3. Goto step 1, 29 more times
4. An incubation at 10°C forever
5. END

The first actual step in the protocol is the incubation at 94°C for 1 minute. There are three scenarios for programming this initial temperature step.

1. If there are no steps displayed:

- **Select <Temp>.**

  A temperature step will be added as the first step in the protocol with a default temperature of 55.0°C and a duration of 30 seconds.

  Proceed to scenario 3 for instructions on editing step parameters.

2. If the first step displayed is a gradient step:

- **Select the gradient step (step 1).**
- **Select <Temp>.**

  This will add a new temperature step to the protocol as step 2 with a default temperature of 55.0°C and a duration of 30 seconds.

  Note: Steps are always added after the step that is currently selected.

- **Select the gradient step (step 1).**
- **Select <Delete Step>.**

  The initial gradient step will be deleted, and the newly added temperature step will be promoted to step one.

  Proceed to scenario 3 for instructions on editing step parameters.

3. If the first step displayed is a temperature step, or if a temperature step was added as indicated in scenarios 1 or 2:

- **Position the cursor (text pointer) in the temperature field of step 1 and select by tapping/clicking once.**

  A flashing insertion point will appear in the temperature field. (See the "Selecting a Step" and "Editing Step Parameters" sections on page 5-20 for additional selection and editing options.)

- **Use the backspace key on the numeric keypad to delete the current temperature if it is not 94.0°C.**

- **Enter 94 from the numeric keypad.**

- **Tap or click once to accept the change.**

- **Position the cursor (text pointer) in the time:minute field of step 1 and select by tapping/clicking once.**

- **Use the backspace key to delete the current value if it is not 01.**

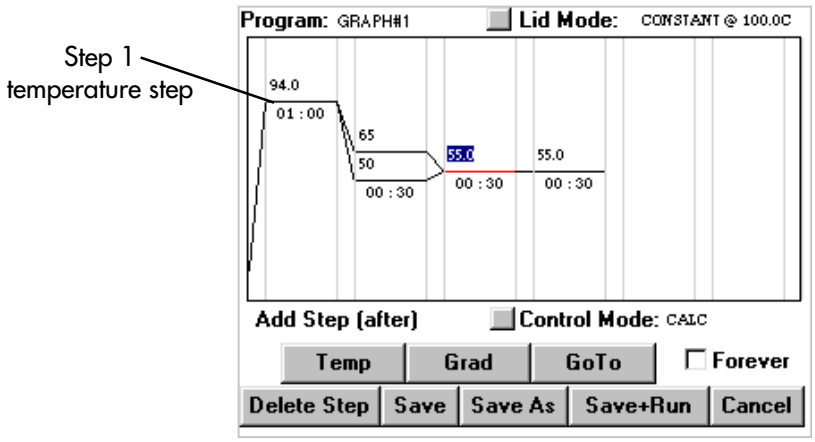
- **Enter 01 from the numeric keypad, and tap or click once to accept the change.**

- **Position the cursor (text pointer) in the time:second field of step 1 and select by tapping/clicking once.**

- **Use the backspace key to delete the current value if it is not 00.**

- **Enter 00 from the numeric keypad, and tap or click once to accept the change.**

Step one of our protocol now consists of a temperature step with an incubation temperature of 94.0 and a duration of 01:00.



Recall that the maximum programmable temperature is 105.0°C and the minimum programmable temperature is 0.0°C in a graphical program. The maximum duration of a temperature step in a graphical program is 99 minutes and 59 seconds or forever.

Step two of our program is also a temperature step, but with an incubation temperature of 92.0°C and a duration of 30 seconds.

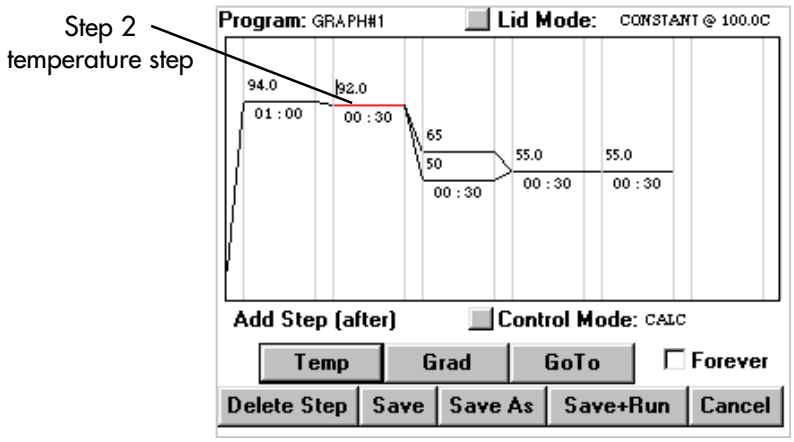
If step two in the displayed protocol is a temperature step, follow the instructions in scenario 3 and in the "Editing Step Parameters" section to alter the parameters of the displayed step.

If step two is not a temperature step:

- **Select step 1.**
- **Select <Temp>.**

A new temperature step will be added after step 1 with the default temperature and time parameters. Follow the instructions in scenario 3 and in the "Editing Step Parameters" section above for information on altering the step parameters.

Step two of our protocol should now specify a temperature incubation of 92.0 for a duration of 00:30.



Entering a Gradient Step

Step three of our program is a gradient step designed to determine the optimal annealing temperature of our oligonucleotide. If step three of the displayed protocol is not a gradient step (two black lines):

- **Select step two.**
- **Select <Grad>.**

A new gradient step should now appear as step three with the default higher temperature limit of 65°C and the default lower temperature limit of 50°C. The default duration is 30 seconds.

The maximum temperature range for a gradient is 24°C and the minimum is 1°C. Fractional degrees are not accepted.

Recall that in our example protocol, the gradient step should specify a range of 45°C to 65°C and a duration of 3 minutes.

- **Position the cursor (text pointer) in the higher limit temperature field and select by tapping/clicking once.**
- **Use the backspace key on the numeric keypad to delete the higher temperature if it is not 65°C.**
- **Enter 65 from the numeric keypad.**
- **Repeat the steps above for the lower temperature limit, entering a value of 45 from the keypad.**

- **Tap or click once to accept the changes to the gradient range.**

  Note: Change both the higher and lower temperatures before accepting the changes to the gradient step to ensure that the temperature differential is not greater than 24°C or less than 1°C.

- **Position the cursor (text pointer) in the time:minute field of step 3 and select by tapping/clicking once.**

- **Use the backspace key to delete the current value if it is not 03.**

- **Enter 03 from the numeric keypad, and tap or click once to accept the change.**

- **Position the cursor (text pointer) in the time:second field of step 3 and select by tapping/clicking once.**

- **Use the backspace key to delete the current value if it is not 00.**

- **Enter 00 from the numeric keypad, and tap or click once to accept the change.**

Entering a Goto Step

Step 4 of our program incorporates a goto step designed to cycle a portion of the program a predetermined number of times. We have chosen to cycle back to step 2 and repeat steps 2 and 3 an additional 29 times. A graphical program can only contain one goto step. This goto step can not be the first or only step in the protocol.

If there is a goto step (blue arrow) in the displayed protocol:

- **Select the goto step.**

- **Select <Delete Step>.**

To add a goto step to our protocol:

- **Select step 3 (the last step to be included in the goto loop).**

- **Select <GoTo>.**

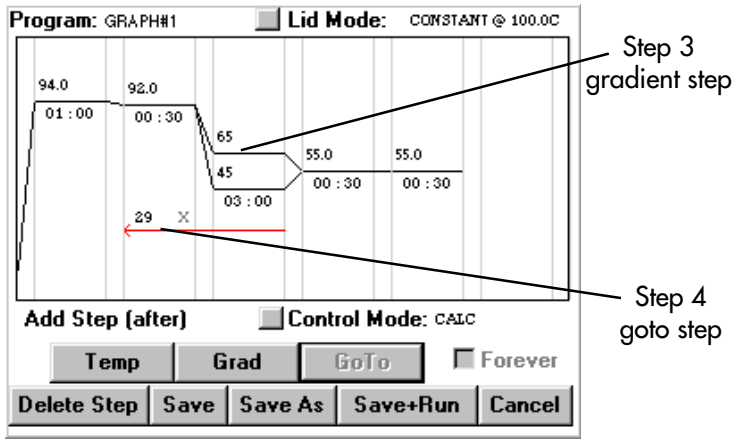
A small red arrow will appear under step 3.

- **Select step 2 (the first step to be included in the goto loop).**

  A red arrow will now extend from the end of step 3 to the beginning of step 2, and the default number of cycles the loop will execute, 25 X, will be displayed.

- **Select the number of cycles and delete using the backspace key.**

- **Enter 29 from the numeric keypad, and tap or click once to accept the change.**

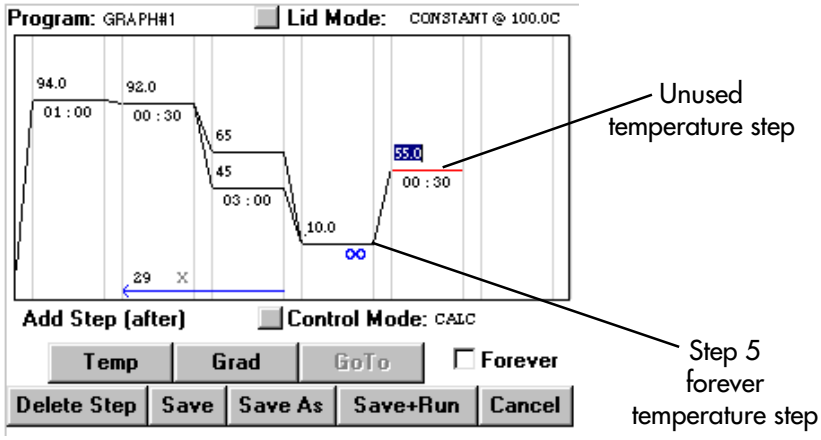


Our program will now run with 30 cycles.

Entering a Forever Incubation

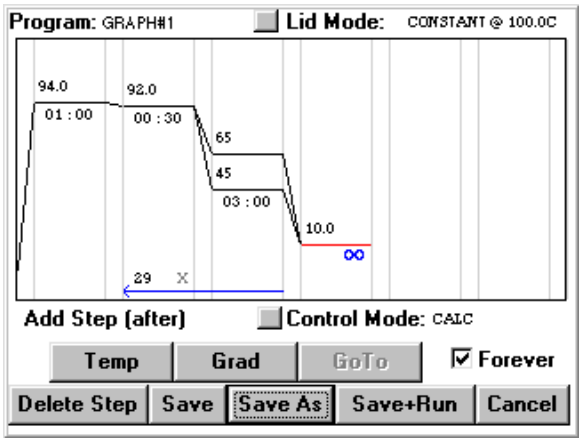
Step 5 of our program is a forever incubation at 10.0°C to help maintain the integrity of our samples until they can be processed. Please note that the instrument can maintain samples at lower temperatures if desired (e.g., 4°C)—but, colder temperatures require considerably more power to maintain, and are unnecessary in most circumstances, in the opinion of MJ Research scientists.

Adding a forever incubation step is identical to programming a temperature step with the exception of selecting the <Forever> box after specifying the incubation temperature. The duration of the step will be displayed as a blue infinity symbol.



If there are any additional steps displayed in the graphical programming window that you do not wish to include in the program, select the step, and then select <Delete Step>. We do not wish to include the temperature step displayed in the fifth temperature/gradient step column.

Our completed program appears as follows:



Select <Save> to save the completed program, GRAPH#1.

Note: If your completed graphical program still bears the name "TEMPLATE", select <Save As> and enter an appropriate name for the program (see the "Using the File Save As Window" section in this chapter). **A graphical program with the default name, "TEMPLATE", can not be run.**

Entering a Program Using Advanced Mode

- **Select <Programs>.**

  Note: As described earlier, this involves positioning the screen cursor over <Programs> with a fingertip on the touch pad and tapping the touch pad once.

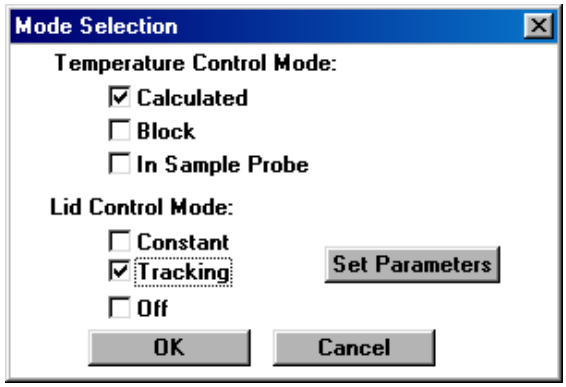
  Drop-down submenus appear, including <Open>, <New>, <Copy>, <Move>, <Delete>, <Delete Folder>, and <New Folder>.

- **Select <New>.**

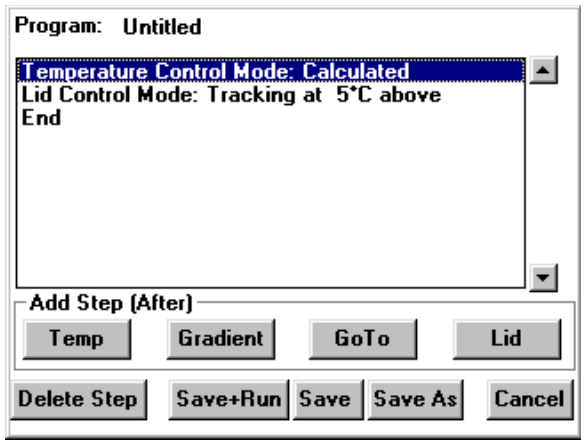
  An additional menu appears allowing you to choose <Advanced Mode> or <Basic Mode>.

- **Select <Advanced Mode>.**

  You are now presented with the Mode Selection window. It is at this point that you will choose the *Temperature Control Mode* and *Lid Control Mode* for the program. Refer to the "Considerations During Program Creation" section earlier in this chapter for information on temperature and lid control modes.



We have decided for the purposes of this example to use *Calculated* for our temperature control mode, and *Constant* for our lid control mode. Refer to the "Using the Mode Selection Window" section above for instructions on entering these choices into our advanced mode program. The Mode Selection window is identical in both graphical and advanced programming modes. However, after selecting <OK> to accept any changes and exit the Mode Selection window, you will return to the advanced programming window.



It is from the advanced programming window that you will add steps using the <Temp>, <Gradient>, <Goto>, and <Lid> options. Additionally, buttons running across the window bottom provide options to <Delete Step>, <Save + Run>, <Save>, or <Save As> programs, and <Cancel> the current programming session.

The <Save> and <Save As> buttons are probably the most important buttons, since a program that is saved can be used or edited at a later date.

- **Select <Save As>.**

  As the File Save As window is identical in both graphical and advanced programming mode, follow the instructions in the "Using the File Save As Window" section above to create a new folder named FOLDER2 and a new program named ADV#1.

  After selecting <OK>, you will be returned to the advanced programming window. The following steps will appear to indicate your progress with the program ADV#1:

  Temperature Control Mode: Calculated

  Lid Control Mode: Constant at 100°C

  End

Entering a Temperature Step

While the program ADV#1 is a bona fide Dyad program, it has no utility. A run of this program will finish immediately after its start, because there are no temperature commands or incubation times to constitute an actual run. Recall again our raw example program:

Use calculated temperature control mode

Use constant lid control mode at 100°C

An initial incubation at 94°C for 1 minute

1. 92°C for 30 seconds
2. Gradient from 45°C to 65°C for 3 minutes
3. Goto step 1, 29 more times
4. An incubation at 10°C forever
5. END

The first actual step in the protocol is the incubation at 94°C for 1 minute. We will use the maximum rate of temperature ramping to this step, and we would like the instrument to beep upon reaching the target temperature.

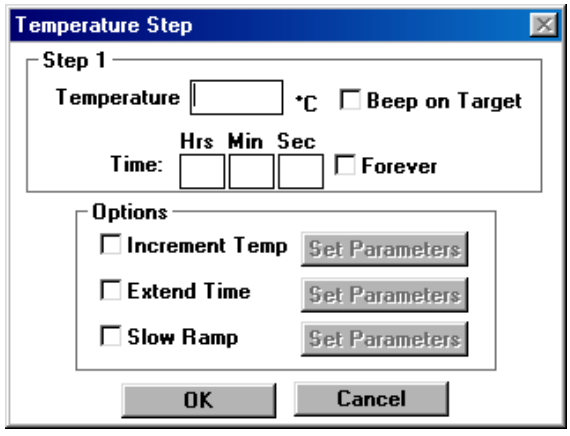
- **Position the cursor over the Lid Control Mode step and tap the touch pad ONCE.**

  Tapping twice will select the step for editing, which will be covered in a later chapter. If you mistakenly select the step for editing, and the Lid Control Mode window appears, just select <Cancel> at the bottom of the window.

  Once the step is selected, any new steps that are added will be inserted after the Lid Control Mode step.

  Tip! Before entering a new step, always select the insertion point first.

- **Select <Temp>.**



The Temperature Step window presents the operator with a number of temperature adjustment options. The *Temperature* and *Time* fields as well as the *Beep on Target* selection option are available at the top of the window. At the bottom, the *Increment Temp*, *Extend Time* and *Slow Ramp* options are available, each with their own <Set Parameters> button. For this particular step, the only parameters that will be set will be the temperature and the incubation time.

Note: The Alpha units can ramp at a maximum of 3.0°C per second. If a slower ramp speed is not entered, this will be the default.

- **Position the cursor over the Temperature field and select.**

- **Enter 94 from the numeric keypad into the field.**

- **Similarly, enter 1 in the *Time:Min* field.**

Additionally, we want the cycler to beep after reaching the target temperature.

- **Select *Beep on Target.***

At this point, in the Temperature Step window, all selections have been made according to our protocol.

- **Select <OK>.**

In the advanced programming window, the program listing now appears as follows:

Temperature Control Mode: Calculated

Lid Control Mode: Constant at 100°C

1. Incubate at 94°C for 00:01:00;

    Beep on Target.

End

The program, if run now, would ramp to 94°C for 1 minute and then end. Let's continue to enter steps and build our program.

The first temperature step in the cycling portion of our program incubates at 92°C for 30 seconds. We would also like the cycler to beep after reaching this temperature.

- **Position the cursor over the appropriate insertion point, if not highlighted already, and select.**

Note: The appropriate insertion point would be selected by highlighting the first step. Insertion will occur AFTER this step.

- **Select <Temp>.**

- Position the cursor over the *Temperature* field and select.

- Enter 92 from the numeric keypad into the field.

- Similarly, enter 30 in the *Time:Sec* field.

  Additionally, we want the cycler to beep after reaching the target temperature.

- **Select *Beep on Target.***

  At this point, in the Temperature Step window, all selections have been made according to our protocol.

- **Select <OK>.**

  In the advanced programming window, the program listing now appears as follows:

  Temperature Control Mode: Calculated

  Lid Control Mode: Constant at 100°C

  1. Incubate at 94°C for 00:01:00

     Beep on Target.

  2. Incubate at 92°C for 00:00:30

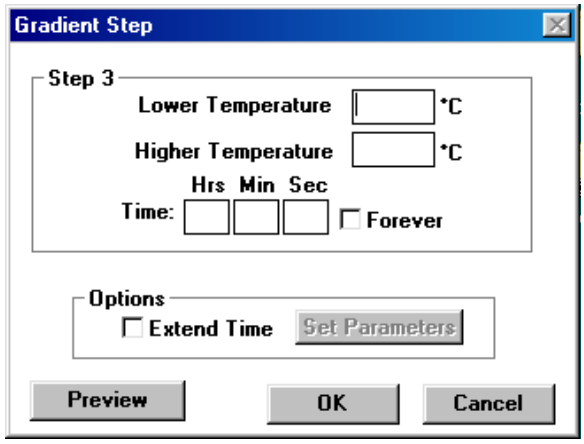
     Beep on Target

  End

  The 92°C denaturation is the first step in the cycling portion of our program. The next step is the gradient step.

- **Highlight Step 2 in the program listing.**

- **Select <Gradient>.**



Entering a Gradient Step

The Gradient Step window indicates the step number and includes fields for specifying the *Lower Temperature* and the *Higher Temperature* of the gradient range as well as the gradient hold *Time*. The maximum gradient range is 24°C and the minimum range is 1°C.

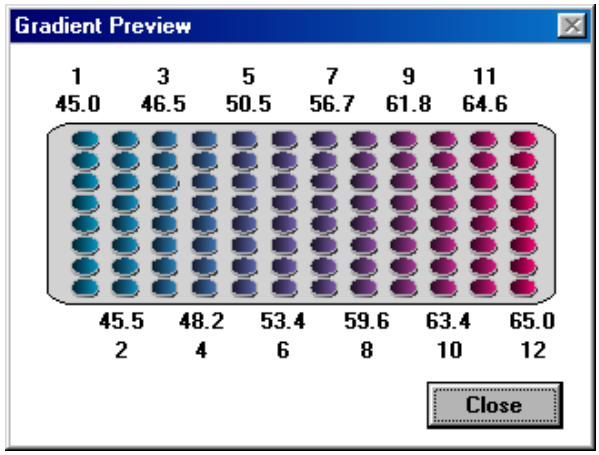
Below, the *Extend Time* option allows for an increase or decrease in incubation time per cycle, similar to the option available for temperature steps.

Since our hypothetical target is 60°C, we will choose 45°C for the lower limit, and 65°C for the higher limit. Additionally, we will enter 3 minutes for the incubation hold time.

- **Select the Lower Temperature field.**
- **Enter 45°C.**
- **Select the Higher Temperature field.**
- **Enter 65°C.**
- **Select the Time:Min field.**
- **Enter 3.**

The newly created gradient can be previewed graphically.

- **Select <Preview>.**



This distribution of temperatures specified by the gradient should be reviewed and any changes to the gradient limits made before a program run. Please note that the gradient temperature differential is not linear, with a broader spread in temperature between the center columns of wells. This is a consequence of the geometry of the Peltier-Joule heaters that underlie the block and is normal. Rest assured that the temperatures displayed are quite accurate for each well in that column (±0.4°C of actual column temperature).

- **Select <Close>.**
- **Select <OK>.**

The program listing should now appear as follows. Since the program step listing field is limited, please use the directional arrow keys to navigate through the steps to review them.

Temperature Control Mode: Calculated

Lid Control Mode: Constant at 100°C

1. Incubate at 94°C for 00:01:00

   Beep on Target

2. Incubate at 92°C for 00:00:30

   Beep on Target

3. Gradient from 45°C to 65°C for 00:03:00

End

As discussed previously, increasing the time per cycle of a temperature step might be desirable in some protocols where extra time is required during later cycles to allow synthesis to be completed.

We will not require such a step in our cycle sequencing protocol, but describe it here for completeness.

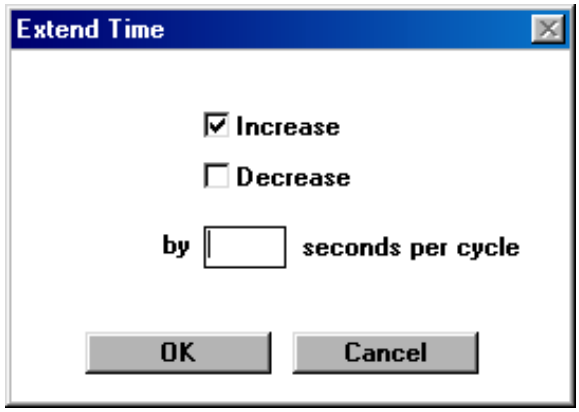
The Extend Time Option

This programming option progressively extends an incubation step with each subsequent cycle. This is typically used during an extension step, to allow for diminishing activity of an enzyme, or to allow an enzyme to do its job among an ever-increasing quantity of product.

Assume, for example, that we required a 60°C step with a 1 minute incubation and that we wished to increase the incubation time by 5 seconds per cycle. In the Temperature Step window, you would implement the following:

- **Enter 60°C in the Temperature field.**
- **Enter 1 in the Time:Min field.**
- **Select Extend Time.**
- **Select <Set Parameters> (for the Extend Time option).**

  The Extend Time window appears.



- **Select Increase.**
- **Position the cursor over the by __ seconds per cycle field and select.**

- **Enter 5 in the field.**
- **Select <OK>.**

This program step, incorporating a per cycle increase in incubation time, would appear as follows:

Incubate at 60°C for 00:01:00

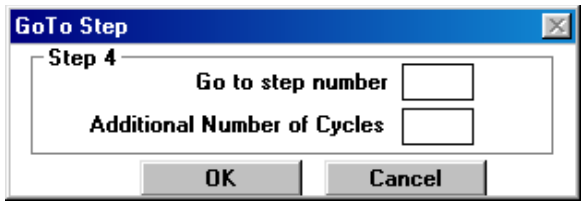
Increase by 5.0 seconds every cycle

This step is not included in our cycle sequencing protocol, so we continue with our next addition.

Entering a Goto Step

As currently entered, our program will run one cycle and then end. What we really want it to do is run 30 cycles total. This involves the insertion of a goto step. Goto steps are useful for cycling your commands a predetermined number of times. We have decided to cycle the steps in ADV#1 twenty nine more times.

- **Select <GoTo>.**



The GoTo Step window appears.

- **Position the cursor in the Goto step number field and select.**
- **Enter 2 in the field.**

Note: We do not want to include step one in the cycling process, as this is our initial denaturation step, and should not be repeated more than once.

- **Position the cursor in the Additional Number of Cycles field and select.**
- **Enter 29 in the field.**
- **Select <OK>.**

The program now appears as follows:

Temperature Control Mode: Calculated

Lid Control Mode: Constant at 100°C

1. Incubate at 94°C for 00:01:00

   Beep on Target

2. Incubate at 92°C for 00:00:30

   Beep on Target

3. Gradient from 45°C to 65°C for 00:03:00

4. Cycle to step 2 for 29 more times.

End

Now the program will run with 30 cycles.

As discussed previously, it may be desirable in some cases to ramp to a temperature at a slower than maximum rate or to include an incremental increase or decrease in temperature per cycle. Some operators may also wish to include instructions in a program to open and close a Power Bonnet™ motorized lid.

A Lid step and the *Slow Ramp* and *Increment Temp* options are not necessary in our example, but the steps necessary to implement them are described below should they be needed in other protocols.

Entering a Lid Control Step

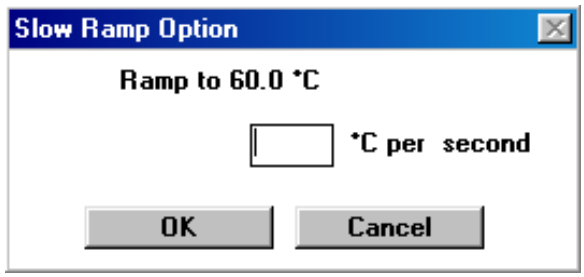
When using a single-block Alpha™ unit fitted with a Power Bonnet™ motorized lid, it may be desirable to include steps in the Dyad program that direct the lid to open or close. This can be particularly useful in robotic installations. To include a lid control step in a program, Select <Lid> in the programming window. Select either *Open Lid* or *Close Lid* in the Lid Control window that appears.

The Slow Ramp Option

Earlier we described the *Extend Time* option for a temperature step, now we will select the *Slow Ramp* option (see the "Choosing a Temperature Ramping Rate" section for additional information). Move from the programming window to the Temperature Step window using the <Temp> button, as before. Enter the appropriate incubation *Temperature* (enter 60 as an example) and *Time* (enter 30 seconds as an example).

- **Select *Slow Ramp*.**
- **Select <Set Parameters> (for the *Slow Ramp* option).**

  The Slow Ramp window appears.



- **Position the cursor over the *°C per second* field and select.**
- **Enter 0.5.**
- **Select <OK>.**

  You would expect the program step as included in a protocol to appear as follows:

  Incubate at 60.0°C for 00:00:30

  Ramp to 60°C at 0.5°C per second

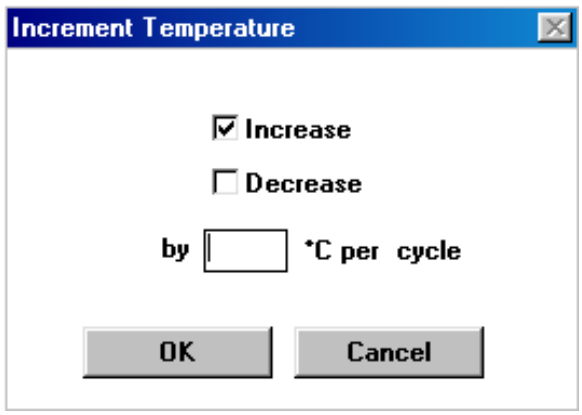
The Increment Temp option

The *Increment Temp* option is useful for modifying a temperature step to allow a "per cycle" increase or decrease of temperature each time the step is executed (see "The Elements of a Program" near the beginning of this chapter for more information).

Move from the programming window to the Temperature Step window using the <Temp> button, as before. Enter the appropriate incubation *Temperature* (enter 60 as an example) and *Time* (enter 30 seconds as an example).

- **Select *Increment Temp*.**
- **Select <Set Parameters> (for the *Increment Temp* option).**

  The Increment Temperature window appears.



- **Select *Decrease*.**
- **Position the cursor over the *by __ °C per cycle* field and select.**
- **Enter 0.2 in the field.**
- **Select <OK>.**

You would expect the program step as included in a protocol to appear as follows:

Incubate at 60.0°C for 00:00:30

Decrease by 0.2°C every cycle

However, such a step is not needed in our protocol, so we will continue onto the next step, the sustained incubation.

We will include an incubation at 10°C, forever, to preserve our sample integrity. The selections are similar to adding a temperature step, with the exception of selecting *Forever*, rather than entering an incubation *Time*.

The final program should appear as follows:

Temperature Control Mode: Calculated

Lid Control Mode: Constant at 100°C

1. Incubate at 94°C for 00:01:00

   Beep on Target

2. Incubate at 92°C for 00:00:30

   Beep on Target

3. Gradient from 45°C to 65°C for 00:03:00
4. Cycle to step 2 for 29 more times.
5. Incubate at 10°C forever

End

The program is now finished. Select <Save> to ensure that your work is preserved.

In Chapter 6, we will learn to edit the various programming steps to include different parameters and in Chapter 7 we will learn how to actually run our program.

6 Managing and Editing Programs

In the previous chapter, various entering and editing features were discussed as they applied to entering a graphical and/or an advanced program. In this chapter, we cover in more depth the options available for the manipulation of existing Dyad programs.

The programming conventions listed in Chapter 5 will also be used here. Please review these before proceeding.

Opening a Program

Once a program has been saved to disk, as the programs GRAPH#1 and ADV#1 were in the previous chapter, it can then be opened for editing or running.

From the Status window menu bar,

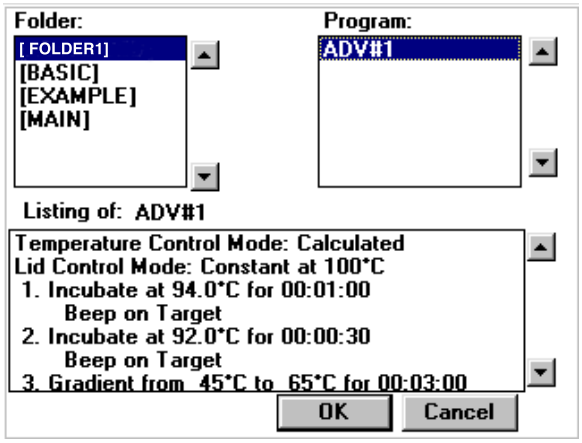
- **Select <Programs>.**
- **Select <Open>.**

  An additional menu appears allowing you to choose <Advanced Mode> or <Basic Mode>.

Opening a Program in Advanced Mode

To open our example advanced program, ADV#1,

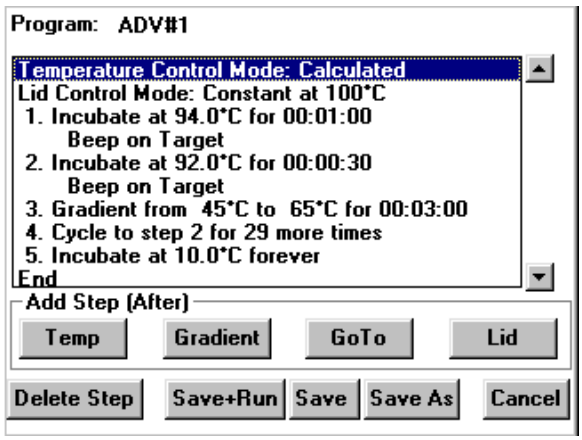
- **Select <Advanced Mode>.**



You are presented with the open program window. In this window, the *Folder* field lists the available folders, and the *Program* field lists all programs available in the currently selected folder. A *Listing of* the steps in the currently highlighted program appears near the bottom of the window.

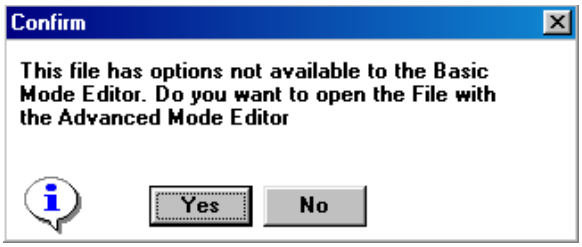
We had previously saved ADV#1 in the FOLDER2 folder.

- **Select the folder FOLDER2.**
- **Select the program ADV#1.**
- **Select <OK>.**



You are presented again with the advanced programming window. It is from this window that steps can be inserted, deleted, or edited.

While all graphical programs can be opened and edited in advanced mode, only a subset of advanced programs can be opened and edited in basic mode. Advanced programs that meet the criteria outlined for graphical programs in the "Types of Programs" section of Chapter 5 can be opened in basic mode. Our advanced program, ADV#1, can not be opened in basic mode because it contains the step modification option, Beep on Target. If an advanced program can not be opened in basic mode, the following message will appear:



Select <Yes> to open the program in advanced mode.

Opening a Program in Basic Mode

You can choose to open and edit a graphical program in either the graphical programming window, or in the advanced programming window. Opening a graphical program in advanced mode is desirable if you wish to add step modification options, incubations below 0°C, or other programming features not available in graphical programs (see the "Types of Programs" section in Chapter 5 for a listing of available program features). However, once advanced-only features are added to a graphical program, the program can no longer be opened or edited in basic mode.

We will open our example graphical program, GRAPH#1, in basic mode. The procedure is similar to that described above for opening a program in advanced mode and is summarized here.

- **Select <Programs>.**
- **Select <Open>.**
- **Select <Basic Mode>.**

  The open program window will appear.
- **Select the folder FOLDER1.**
- **Select the program GRAPH#1.**
- **Select <OK>.**

  GRAPH#1 will be displayed in the graphical programming window.

Editing a Program

Recall our general program.

Temperature Control Mode: Calculated

Lid Control Mode: Constant at 100°C

1. Incubate at 94°C for 00:01:00

   Beep on Target (advanced only)
2. Incubate at 92°C for 00:00:30

   Beep on Target (advanced only)
3. Gradient from 45°C to 65°C for 00:03:00
4. Cycle to step 2 for 29 more times.
5. Incubate at 10°C forever

End

After running the above program, analysis of the resulting sequencing data indicated that 60°C was the best annealing temperature. We would like to change step 3 from a gradient step into a temperature step.

Further, step 2 includes a 30 second incubation that we wish to change to 25 seconds.

Editing a Graphical Program

As graphical programming (discussed in Chapter 5) involves essentially editing a TEMPLATE program, we will only briefly discuss the specifics of editing a preexisting graphical program here. Please refer to "Entering a Program Using Graphical Mode" in Chapter 5, specifically, "The Graphical Programming Window" section for more information.

To replace step 3, the gradient step, with a temperature incubation at 60°C, first open the program GRAPH#1 in basic mode as described above.

- **Select the gradient step.**
- **Select <Delete Step>.**
- **Select step 2, the temperature incubation at 92°C.**

  Recall that new steps are added AFTER the selected step.

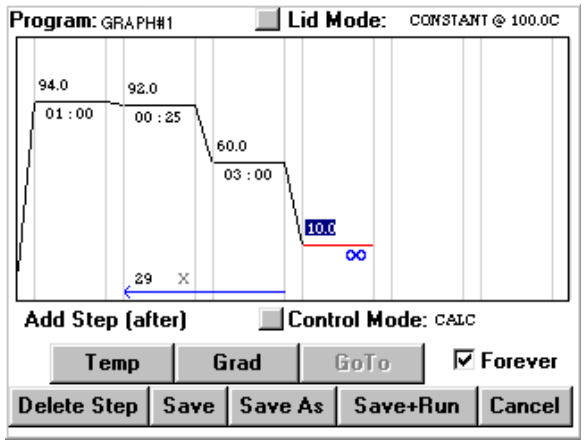
- **Select <Temp>.**

  The gradient step has now been replaced with a default temperature step. Follow the instructions in Chapter 5 for entering temperature step parameters.

To decrease the incubation time of step 2 from 30 seconds to 25 seconds:

- **Select the time field of step 2.**
- **Delete 30 and enter 25 using the numeric keypad.**
- **Tap or click once to accept the change.**

Our example program, GRAPH#1, now appears as follows:



Editing an Advanced Program

Highlighting and Selecting Program Steps

Several important factors determine step selection:

- The insertion point for new steps is AFTER the step highlighted in the program listing. This highlighting is accomplished with a SINGLE tap or click.
- Double tapping/clicking on a program step will immediately open the appropriate step-editing window for the highlighted step. The windows for editing are the same as those for step creation.
- Selection can also be done via the left touch pad button.

  In order to insert or edit steps, the Dyad user should become familiar with these conventions. By default in this manual, we use the single touch pad tap/ mouse click to highlight a step, and a double tap/click to select it.

Deleting a Step

The <Delete Step> button will allow us to delete a program step. To begin the process of changing step 3 of our advanced program, ADV#1, into a temperature step:

- **Highlight step 3 by positioning the pointer over the first line of step three and tapping once.**
- **Select <Delete Step>.**

  The gradient step will be deleted and the following steps renumbered. For example, the go to step, previously step 4, will become step 3.

Inserting a Step

Now we will insert an annealing temperature step.

- **Highlight step 2**.

  Recall that new steps are inserted AFTER the highlighted step.

- **Select <Temp> from the advanced programming window.**

  The Temperature Step window appears. Select an incubation *Temperature* of 60°C, a hold *Time* of 3 minutes, and the *Beep on Target* option. Enter parameters as described in Chapter 5.

  Your program should appear as follows.

  Temperature Control Mode: Calculated

  Lid Control Mode: Constant at 100°C

  1. Incubate at 94°C for 00:01:00

     Beep on Target

  2. Incubate at 92°C for 00:00:30

     Beep on Target

  3. Incubate 60°C for 00:03:00

     Beep on Target

  4. Cycle to step 2 for 29 more times.

  5. Incubate at 10°C forever

  End

Editing a Step

Step 2 of the program ADV#1 includes a 30 second incubation. We wish to change that incubation to 25 seconds.

- **Select Step 2 by double-clicking.**

  You are presented with the Temperature Step window. The *Temperature* field shows 92°C, and the *Time* field shows 30 seconds.

- **Select the *Time:Sec* field.**

- **Change the time from 30 to 25.**

- **Select <OK>.**

Now the Program Editing window lists the program steps as the following:

Temperature Control Mode: Calculated

Lid Control Mode: Constant at 100°C

1. Incubate at 94°C for 00:01:00

   Beep on Target

2. Incubate at 92°C for 00:00:25

   Beep on Target

3. Incubate 60°C for 00:03:00

   Beep on Target

4. Cycle to step 2 for 29 more times.

5. Incubate at 10°C forever

End

Note: You may need to utilize the directional arrow keys to view all steps.

File Utilities

Once you have edited a program, any number of manipulations can be used to archive it for later use, including saving, copying, deleting and moving. You can also delete a folder. These functions, with the exception of saving, can be accessed from the <Programs> drop down menu on the Status window menu bar.

Saving an Edited Program

The decision required here is whether to save the program under the same, or different, filename. In Chapter 5, we discussed the <Save As> button, which allows the creation of a new filename.

In this instance, we will simply save the edited files under the same name.

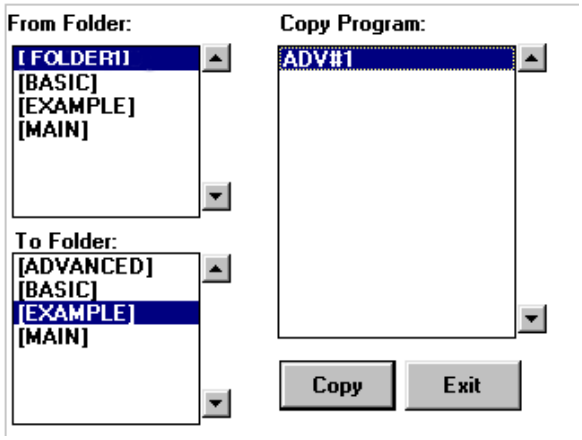
- **Select <Save>.**

  Your file can now be selected and reviewed for further editing.

  Please note that in the advanced programming window, utilizing the <Save> feature will bring you to the Status window after implementation, whereas, the <Save As> feature will bring you back to the advanced programming window. Therefore, if you plan on continuing to edit the file, <Save As> would be the simpler choice.

Copying a Program

- **Select <Programs> in the Status window.**
- **Select <Copy> from the drop-down menu.**



Once you have created a number of programs, you may want to create separate folders to organize them. Perhaps you will use separate folders for different users, or experimental series. To copy a program:

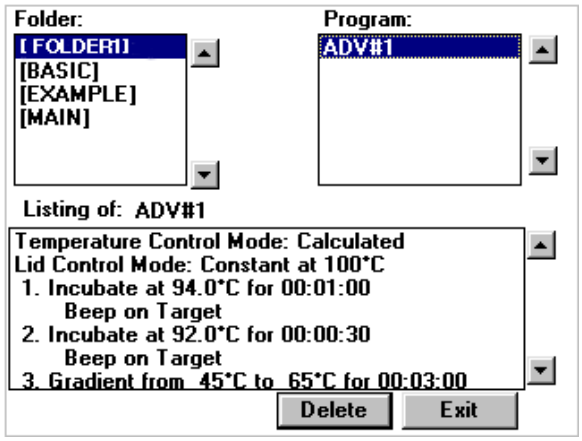
- **Highlight the *From Folder* and *To Folder* locations for the copy.**
- **Highlight the program in the *Copy Program* list to be copied.**
- **Select <Copy>.**

  The program now resides in the destination folder.

- **Select <Exit> to return to the Status window.**

Deleting a Program

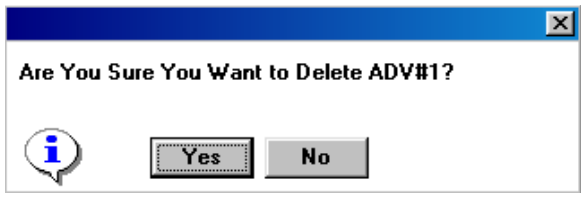
- **Select <Programs> in the Status window.**
- **Select <Delete> from the drop-down menu.**



Important!: Use caution when deleting programs, as deletions are irreversible, and the delete program window is very similar to the open program window. We provide a convenient program listing at the bottom of the window so that you can determine whether you truly wish to delete the program.

- **Highlight the Folder containing the program to be deleted.**
- **Highlight the Program.**
- **Select <Delete>.**

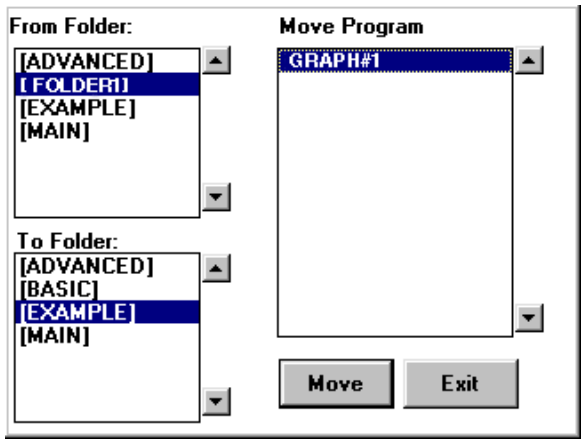
Before a program is deleted, you are presented with a confirmation screen asking you to verify the deletion.



If you wish to delete the program, select <Yes>. If you want to keep your newly created program, select <No>.

Moving a Program

- **Select <Programs> in the Status window.**
- **Select <Move> from the drop-down menu.**

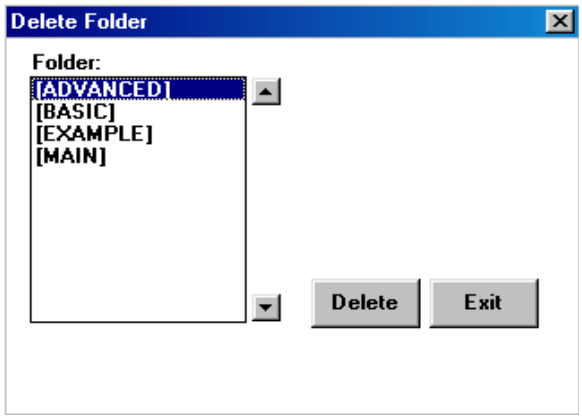


Moving is the same as copying (described above), with one distinction: only one copy of the program is maintained in the *To Folder*. The copy in the *From Folder* is deleted. You will not be prompted with a verification step for this move, so exercise some caution.

Deleting a Folder

No command set would be complete without a folder maintenance window. Folders must be empty before deletion.

- **Select <Programs> in the Status window.**
- **Select <Delete Folder> from the drop-down menu.**
- **To delete a folder, highlight it and select <Delete>.**



You will be presented with a confirmation screen asking you to verify the deletion. Select <Yes> to delete the folder.

In this chapter, we have utilized editing tools to add and subtract steps from our example programs. In addition, we learned to utilize file and folder tools to maintain our program lists.

In subsequent Chapters, we will learn more about the menu bar of the Status window, and how to navigate the utilities and functions located there. We will also learn how to run our new program.

7 Running Programs

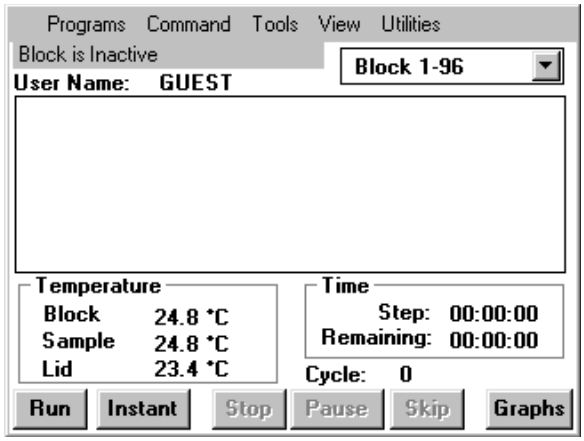
In Chapter 5, we translated an experimental protocol into functional Dyad programs. In Chapter 6, we edited program steps. In this chapter, we explore the implementation of the Dyad programs, and the instant incubation feature.

Using the Instant Incubation Feature

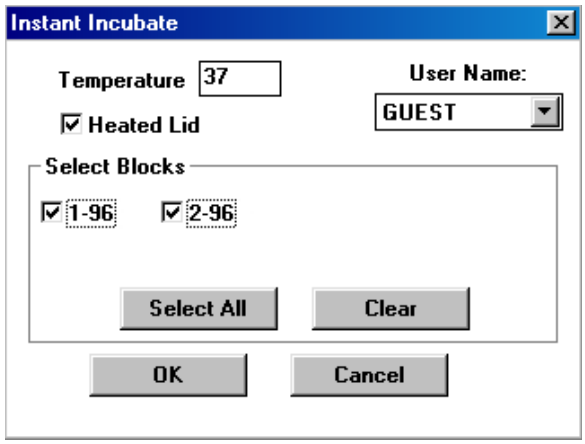
The DNA Engine Dyad cycler can also be quite useful as an "instant" constant-temperature incubator, with a range from –5.0°C to 105°C (4°C to 100°C with the Twin Towers® block). This feature can be used for performing ligations, digestions, etc.—or with slides, overnight, humidified hybridizations.

Note: The Twin Towers block can be used as a humidified chamber for steady-state incubations (e.g., hybridizations, color-development reactions). To humidify a block, push one laboratory tissue into the bottom slot and inject 1ml of deionized water onto it. See the *Twin Towers Operations Manual* for complete instructions.

To initiate an instant incubation from the Dyad Status window,



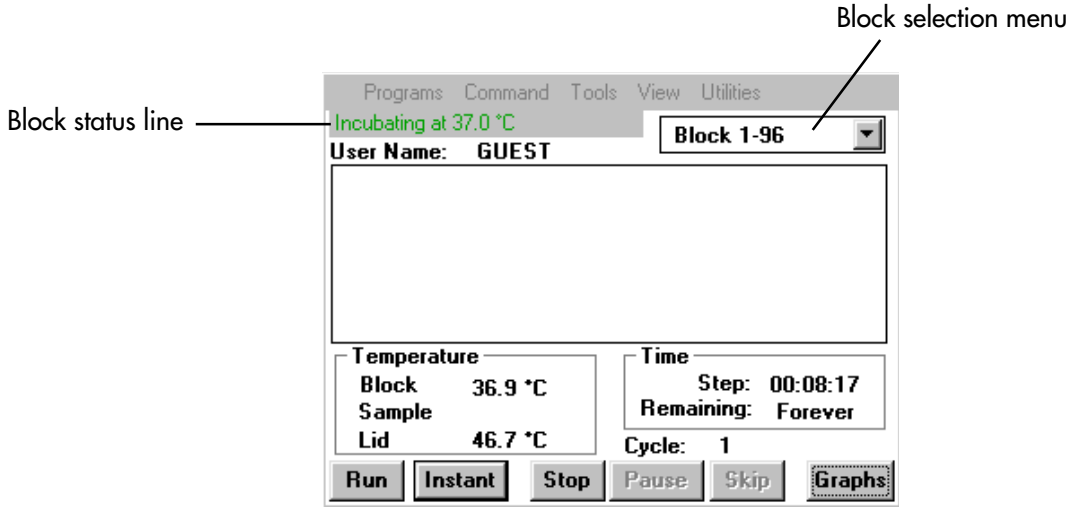
- **Select <Instant> at the bottom of the window or from the <Command> menu.**



- **Select the Temperature field and enter the desired temperature.**
- **Select Heated Lid if you are incubating at a high temperature and wish to minimize condensation (refer to the "Sealing with the Hot Bonnet Lid" section in Chapter 4 for additional information on using the heated lid).**
- **Select the desired block(s).**
- **Select <OK>.**

The block(s) will now incubate at the desired temperature.

The Status window will display the status of the selected block. Use the block selection menu to select a block.



To stop the incubation, select the appropriate sample block from the block selection menu. Select <Stop> at the bottom of the window. A confirmation window will appear asking you to verify termination of the instant incubation. Select <Yes> to stop the incubation or <No> to continue.

Running Programs

Note: The Operations Chapter (Chapter 4) provides information on running programs with respect to the operation and use of Alpha units. For example, a program containing a gradient step will only run in 96-well Alpha units. Please review this chapter for additional technical detail, to ensure that the program you load and run is an appropriate match for your Alpha units.

To run a program displayed in either the advanced programming window or the graphical programming window, select <Save+Run>. Recall that prior to running a newly created graphical program, the program must be assigned a name other than the default name, "TEMPLATE", by performing a save as. **Graphical programs with the default name, "TEMPLATE", can not be run.**

The Run Program window will appear allowing you to specify the block on which the protocol should be run, and the calculated control parameters for the run (if applicable) as described below.

To run previously created programs (see Creating Programs, Chapter 5 and Managing and Editing Programs, Chapter 6), you must first load them into memory.

From the Status window,

- **Select <Run> at the bottom of the window or from the <Command> menu.**

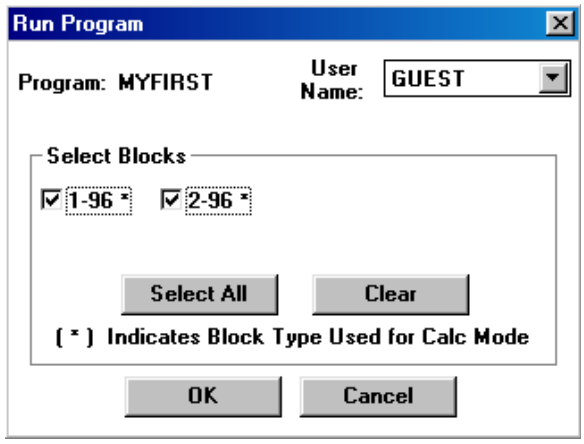
- **From the open program window that appears, select the desired Folder and Program.**

  Double check the program listing to ensure that the listed steps are consistent with the desired program.

- **Select <OK>.**

You are now presented with the Run Program window.

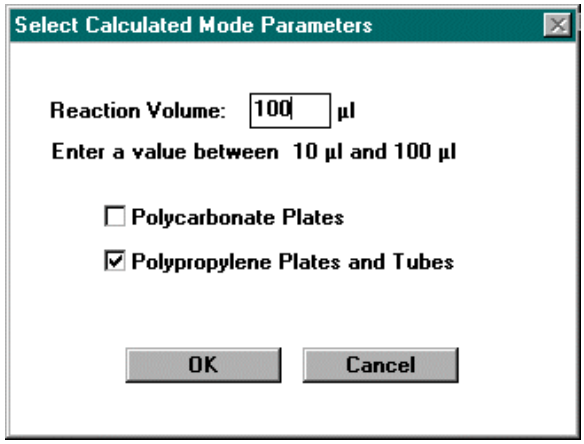
The Run Program window



The Run Program window allows you to select the Block(s) that you wish to run your protocol on. You can select a single block or all blocks (based on block compatibility with your program).

If you are preparing to run a program using calculated temperature control, selecting <OK> will display a Select Calculated Mode Parameters window that is appropriate for the type of program and Alpha unit that you are using. Parameters entered here will allow precise temperature calculations by the Dyad cycler for your specific protocol and Alpha unit.

For example, for a 96-well Alpha unit, the following selection window will appear:



- **Enter the reaction volume of your samples and select the type of reaction**

vessels used.

- **Select <OK> to initiate the run.**

Refer to the "Running Multiple Programs" section at the end of this chapter for information on simultaneously running multiple programs.

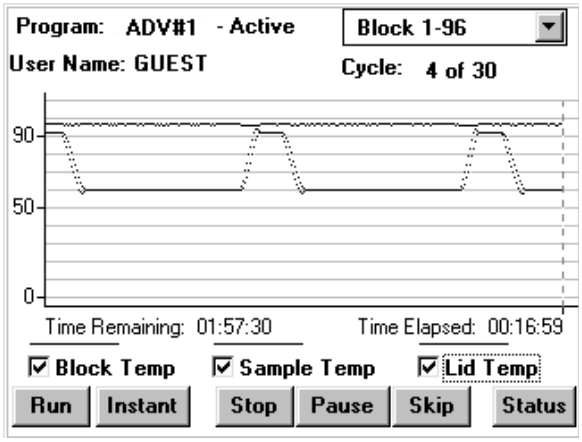
During the Run

Run Status

Information in the *Temperature*, *Time,* and *Cycle* fields of the Status window will indicate that your program is running. To graphically display the run conditions, go to the Graphs window.

- **Select <Graphs> at the bottom of the window or from the <View> menu.**

  To graph the *Block Temp*, *Sample Temp*, and/or *Lid Temp* over time, select the appropriate options near the bottom of the Graphs window. The estimated *Time Remaining* in the program and the amount of *Time Elapsed* since the program was initiated are also indicated.

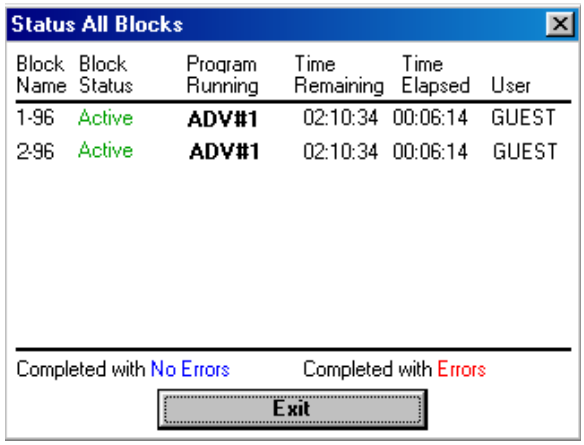


- **Select <Status> to return to the Status window.**

  The Status window will display *Block*, *Sample*, and *Lid* temperatures correlating to the program running on the block chosen in the block selection menu. These temperatures represent real-time readings and correspond to the values represented graphically in the above window. The time remaining in the current *Step*, and the *Remaining* time in the program are displayed along with the current *Cycle* number.

Tip: Recall that convenient and rapid toggling between the Status and Graphs windows can be achieved by selecting the button in the lower right of the Status and Graphs windows respectively.

To simultaneously view the status of all blocks including the *Block Name*, *Block Status*, the name of the *Program Running*, the *Time Remaining* in the program, the *Time Elapsed*, and the *User* name, select the <View> menu and then <Status All> from the drop-down list.



If the Block Status indicates that the program was completed with errors, select <Error Log> from the <View> menu to view error messages.

To view a run log including the date and time that a program was initiated by a user, select <Cycler Log> from the <View> menu.

Terminating a Run

To terminate a run prior to completion, select the appropriate block from the block selection menu in the Status window and select <Stop>. Alternatively, select the <Command> menu in the Status window, and then select <Stop> and either <All> to terminate all programs running on all blocks, or select the desired block from the drop-down list.

Pausing/Resuming a Run

To merely pause a run, select the appropriate block from the block selection menu in the Status window and select <Pause>. Alternatively, select the <Command> menu in the Status window, and then select <Pause/Resume> and either <Pause All> to pause all programs running on all blocks, or select the desired block from the drop-down list.

To resume a run, select <Resume> from the bottom of the Status window or select <Command>, <Pause/Resume>, and either <Resume All> or select the desired block from the drop-down list.

Skipping a Step

To skip to the next step in a running program, select <Skip> at the bottom of the Status window. A confirmation screen will ask you to confirm the skip, select <Yes> to skip to the next step in the protocol displayed in the Status window.

Inaccessible Features

Considerations must also be made for the compatibility of the program with the installed Alpha units. The Run Program window will display one or more "grayed-out" Alpha units in the *Select Blocks* section if there is an incompatibility with the program in queue, or if an Alpha unit is currently unavailable due to a protocol that is already running.

Some scenarios which may be the cause of inaccessible features or display changes include:

Block control: A sample temperature will not be displayed.

Probe control: Sample temperature will be replaced with Probe temperature. Additionally, programs with lid temperature steps will not run with probe control.

Gradient step: Alpha units other than 96-well Alpha units will be "grayed-out".

Lid step: Alpha units will be "grayed-out" if a lid step is included in the protocol, but there is no Power Bonnet lid installed.

Lid mode: Alpha units will be "grayed-out" if the lid mode is not set to OFF when using a block with no lid such as a Twin Towers Alpha unit.

When encountering an inaccessible feature or block, please review your program with the installed Alpha units to determine if an incompatibility is present.

Running Multiple Programs

One particularly useful feature of the Dyad cycler is the ability to run several programs at once on different blocks. For example, in a Dyad cycler setup with two 96-well Alpha units, a gradient for optimizing annealing temperature can be run in one Alpha unit, whereas a typical experiment without a gradient can be run in the other Alpha unit, ***simultaneously.***

Before running multiple protocols, considerations should be made as to the compatibility of the program with the available Alpha units. Please review Chapter 4: *Operation* for a more complete treatment of Alpha units.

To run multiple programs, first choose an available block from the block selection menu in the Status window. Available blocks will show a "Block is Inactive" message just above the *User Name* field. If there are no available blocks, the "No blocks available" message window appears. Select <Run> and follow the instructions in the "Running Protocols" section in this chapter to initiate an independent run.

To summarize, we have learned how to run programs on the Dyad cycler. We have learned how to select our options based on the type of Alpha unit and sample vessels used in our experiment. We have learned how to terminate a program run. Finally, we have learned how to use the most versatile feature of the Dyad cycler by running multiple program simultaneously.

In subsequent chapters, we will discuss the remaining menu and submenu items, particularly the Utilities.

8 Using the Utilities

In previous chapters, you've learned how to install and operate the Dyad cycler, as well as write and execute programs. In this chapter, the various functions of the <Utilities> submenu will be discussed. The <Utilities> submenu rounds out the capabilities of the Dyad cycler, providing access to user name control, remote command mode, and the date and time settings.

The <Utilities> submenu is selectable from the Status window.

Here is a review of the available submenus.

<About>

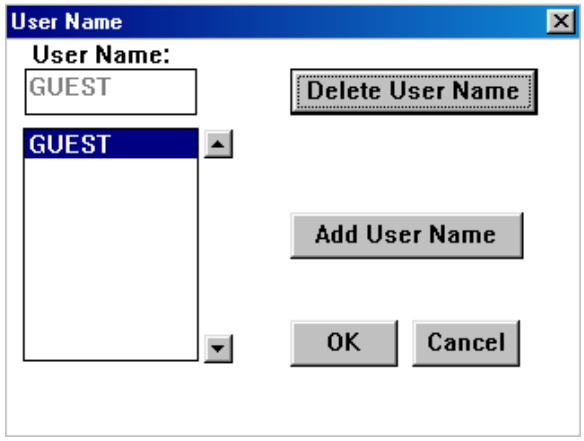
When this item is selected, a screen like the following will appear:

This screen indicates the Dyad system software version. The software version information on this screen can be used to determine if an update is necessary.

Select <OK> to exit from this screen.

<User Name>

When this item is selected, the following screen will appear:



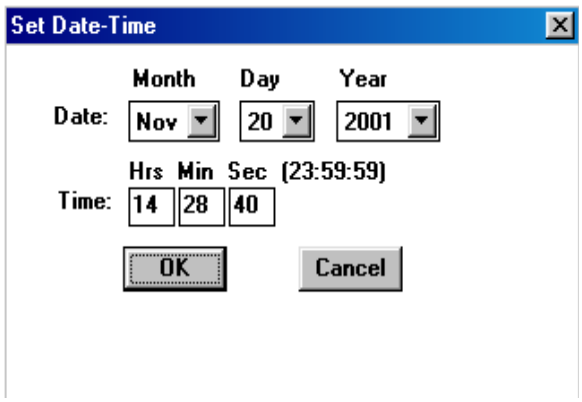
By selecting <Add User Name>, the virtual keyboard will appear allowing users to enter their name prior to program execution. In this manner, any potential user can determine the "owner" of a block (the person running a program) as their name will be displayed when the block is selected in the Status window.

To delete a user name, highlight the name and select <Delete User Name>.

Select <OK> to exit from this screen.

<Set Date/Time>

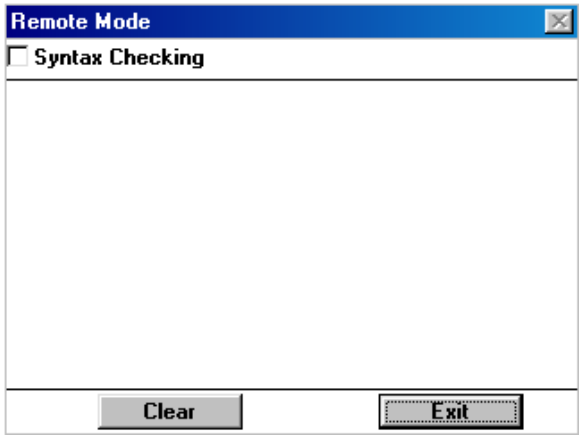
Choosing this option will allow the setting of the Dyad system time.



Enter the appropriate information in the fields provided and select <OK>.

<Remote>

To operate a Dyad cycler in remote mode (i.e., from a desktop computer), select <Remote> from the <Utilities> menu. The Remote Mode window will appear on the screen.



The only option available is a check box for enabling syntax checking. This will filter out any commands that do not conform to the PTC Remote Command Set syntax. Attach a null modem serial interface cable (MJR#02371 or equivalent) to the RS-232 port connector on the back of the Dyad cycler. Connect to either COMM1 or COMM2 on a PC, or the serial port on a Mac. The Dyad cycler is now ready to be run in remote mode.

Important! *Do not exit remote mode while a protocol is running!* If a protocol is loaded and run remotely, ending the remote session by exiting the Remote Mode window will cancel this protocol. A confirmation screen will ask you to confirm your intent to exit the Remote Mode window.

Log on to www.mjr.com for the latest syntax of the remote command set.

<Auto Remote>

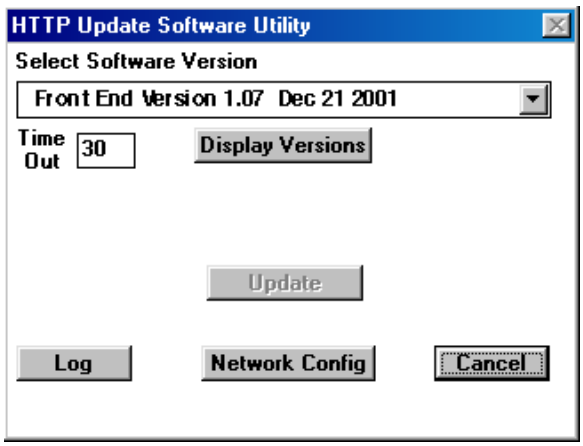
Select <Auto Remote> from the <Utilities> menu to direct the Dyad cycler to automatically enter remote mode upon subsequent power up. This feature is particularly useful if you consistently control the Dyad cycler using a desktop computer. It eliminates the need to select <Remote> from the <Utilities> menu after every power up.

To disable the auto remote feature, first select <Exit> to exit remote mode (see the warning in the <Remote> section above). The cycler will return to the standard operation mode, and the Status window will be displayed. Select <Auto Remote> from the <Utilities> menu such that a check mark no longer appears in front of the Auto Remote option. The cycler will now power up in standard operation mode.

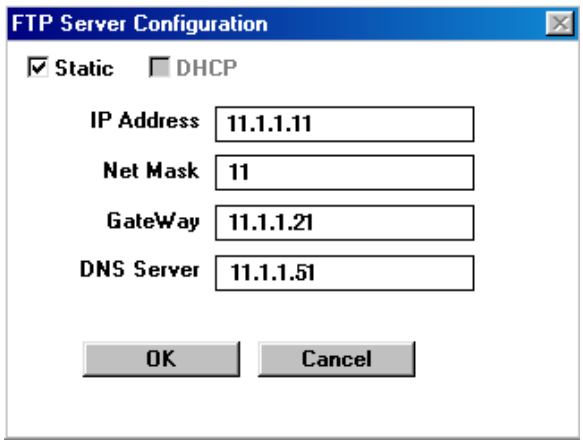
<Update Soft> & <Network Config>

These utilities allow you to update both the software controlling the Dyad cycler's hardware (i.e., engine software), and the user-interface/programming software (i.e., front end software) by establishing an internet connection and directly downloading any new software upgrades or versions. If you are unsure of the current version of engine and/or front end software installed on your Dyad cycler, use the About utility as described in the beginning of this chapter to view the current software versions. Front end software version 1.07 or greater is required to use the <Update Soft> and <Network Config> utilities.

To view the available software upgrades and/or perform an upgrade, begin by establishing internet access via an Ethernet 10BaseT connection. Connect the ethernet cable to the ethernet port located at the rear of the Dyad cycler (see figure 2-3). Then, select <Update Soft> from the <Utilities> menu.



The HTTP Update Software Utility window appears. Begin by specifying the type of connection that you have to the internet by selecting <Network Config>. The FTP Server Configuration window appears. You can also directly access this window by selecting <Network Config> from the <Utilities> menu.



If you have a static internet connection, i.e., the IP address does not change, select the *Static* option. Enter your *IP Address*, *Net Mask*, *GateWay*, and *DNS Server* information using the numeric keypad. If you lack this information, please contact your network administrator.

If you have a dynamic internet connection, i.e., a different IP address is assigned each time you access the internet, select the *DHCP* (Dynamic Host Configuration Protocol) option. The *IP Address* and the other required fields should populate automatically.

If you are unsure of the type of internet connection available to you, please contact your network administrator.

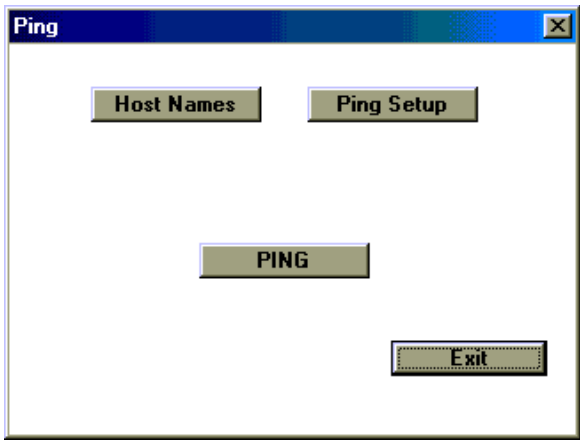
Once you have finished configuring your network connection, select <OK>. A pop-up window will appear directing you to power cycler the instrument in order to implement the connection information.

To update the engine or front end software:

- Select <Update Soft> from the <Utilities> menu to access the HTTP Update Software Utility window.
- Select <Display Versions> to view the available software upgrades.
- Click on the arrow button to the right of the Select Software Version field to display the available versions.
- Select the software that you wish to download.
- Select <Update>.
- A confirmation screen will appear asking you to confirm your desire to update the software. Select <Yes> to proceed with the update.
- A confirmation screen will advise that the update has been completed. If any errors occurred during the update, select <Log> to view the error log. If the update was successfully completed, restart the Dyad cycler.
- Select <About> from the <Utilities> menu to view the currently loaded software version and confirm that the software update has been successfully completed.

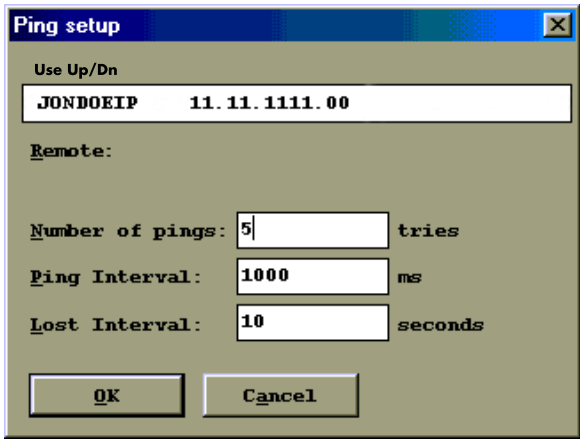
<Ping>

This utility can be used to verify that the ethernet software and connection are properly functioning. Select <Ping> from the <Utilities> menu.



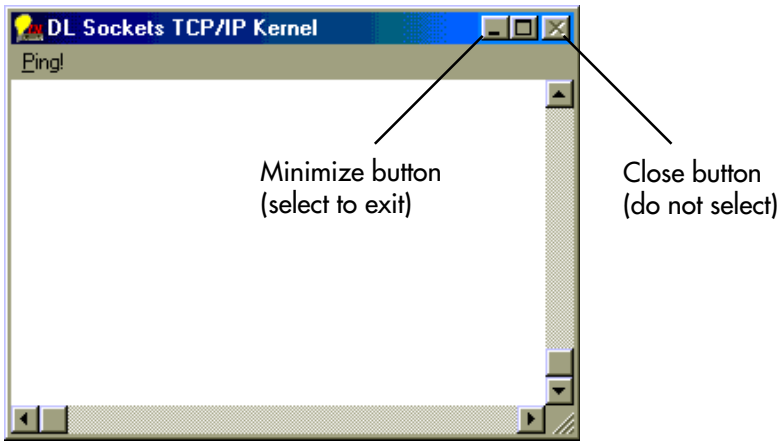
In the Ping window, begin by selecting <Host Names> and entering the IP address of the computer that you wish to ping. All host names must be one word (i.e., no spaces).

Then, select <Ping Setup> from the Ping window.



Select a host name using the up/down cursor keys. Enter the number of times that you wish to ping the host, the interval between pings (ms), and the lost interval, the amount of time (sec) before the request times-out. Select <OK> to return to the Ping window.

Select <PING> to run the ping program. The results will be displayed in the DL Sockets TCP/IP Kernel window. A tally at the bottom of the window will list the total number of ping attempts, the number and percentage of pings received from the host, and the number and percentage of pings lost (i.e., not received from the host).



Select <Ping!> to repeat the ping, or select the minimize button to exit the window.

Note: Do not exit this window by using the close button. If you inadvertently exit using the close button, an error will appear directing you to power cycle the instrument.

Additional Utilities

The <Password> and <Service> utilities are intended for use by MJR personnel only.

Other functions may be added to the Dyad system software in future updates. Please follow www.mjr.com for update information.

Transferring Program Files

During the initial boot-up sequence of the Dyad cycler (see the "Turning the DNA Engine Dyad Cycler On" section in Chapter 4), several options are available for transferring program files. Options *2 Send Files* and *3 Receive Files* can be used to establish a Dyad cycler to Dyad cycler transfer of all program files in all folders.

To perform a Dyad cycler to Dyad cycler file transfer:

1. Use a standard DB9 null-modem cable to connect the Dyad cyclers via their RS-232 ports.

2. During the boot-up sequence of the sending instrument, select the *Send Files* option by entering 2 on the numeric keypad.

3. During the boot-up sequence of the receiving instrument, select the *Receive Files* option by entering 3 on the numeric keypad. The instrument will indicate that it is *Ready* to accept files.

4. To execute the file transfer, select *Proceed (& Exit when completed)* by entering 1 on the numeric keypad of the sending instrument. Once the transfer is complete, the *TRANSFER COMPLETE* message will be displayed and the instrument will proceed with the boot sequence.

9 Maintenance

Cleaning the DNA Engine Dyad Cycler

Cleaning the Chassis and Blocks

Clean the outside of an Alpha unit or the cycler chassis with a damp, soft cloth or tissue whenever something has been spilled on it or when the chassis is dusty. A mild soap solution may be used if needed. Allowing major buildup of laboratory dust or other contaminants may affect the performance of the cycler or Alpha units, as well as, the outcome of your experiments. As with any thermal cycling experiment, a reasonably clean, contaminant free environment is recommended.

For particularly sensitive reactions, where contamination could confound results, we recommend use of an MJ Research Cleanbox or equivalent, which utilizes a UV lightsource to inactivate extraneous DNA.

Three models of cleanbox are offered by MJ Research:

CBX-0750 75cm wide, single door, 120V
CBX-0900 90cm wide, single door, 120V
CBX-0120 120cm wide, dual doors, 120V

To clean block wells, use swabs moistened with water, 95% ethanol, or a 1:100 dilution of bleach in water (see the *Twin Towers Operations Manual* for instructions on cleaning the Twin Towers slide slots). If using bleach, swab wells with water afterward to remove all traces of bleach. Clean spilled liquids out of the block as soon as possible; dried fluids can be difficult to remove. Do not clean the block with caustic or strongly alkaline solutions (e.g., strong soaps, ammonia, bleach at a higher concentration than specified above). These will damage the block's protective coating, possibly causing electrical shorting.

If you use oil in the block (a practice not recommended by MJ Research, Inc.; see "Using Oil to Thermally Couple Sample Vessels to the Block," in Chapter 4), clean the wells whenever the oil has become discolored or contains particulate matter. Use a swab to determine whether cleaning is needed. Clean the block with 95% ethanol as described above. **Oil buildup must be prevented.** Old oil harbors dirt, which interferes with vessel seating and diminishes thermal coupling of sample vessels to the block.

⚠ **Caution:** Do not pour any cleaning solution into the block's wells and then heat the block, in an attempt to clean it. Severe damage to the block, the heated lid, and the chassis may result.

Cleaning the Air Vents

Clean the air intake and exhaust vents with a soft-bristle brush, a damp cloth, or a vacuum cleaner whenever dust is visible in them. The air intake vents are located on the bottom, lower front edge, and back of the machine; the air exhaust vents are located on both sides (see figures 2-1, 2-3, and 2-4). If these vents become clogged with dust and debris, airflow to the Alpha unit's heat sink is hampered, causing performance problems related to overheating. The air intake vents are particularly likely to collect dust since their holes are much smaller than those of the air exhaust vents, to prevent debris from entering the instrument.

✔ **Tip:** To prevent problems with overheating, institute a regular program of checking for dust buildup, particularly for robotics installations.

Cleaning Radioactive or Biohazardous Materials From the Block

When cleaning machines that have been running radioactive or biohazardous reactions, consult your institution's radiation safety officer or biosafety officer regarding cleaning methods, monitoring, and disposing of contaminated materials.

Changing the Fuses

The circuits in the DNA Engine Dyad cycler are protected by two fuses (6.3A fast-acting, 5 x 20mm). When a fuse blows, the DNA Engine Dyad cycler immediately shuts down and cannot be turned back on. The machine records the event as a power loss. If a protocol is running when a fuse blows, the machine will resume the run when the fuse is replaced and the power restored.

⚠ **Warning:** The DNA Engine Dyad cycler incorporates neutral fusing, which means that live power may still be available inside the unit even when a fuse has blown or been removed. Never open the Dyad base. You could receive a serious electrical shock. Opening the base will also void your warranty.

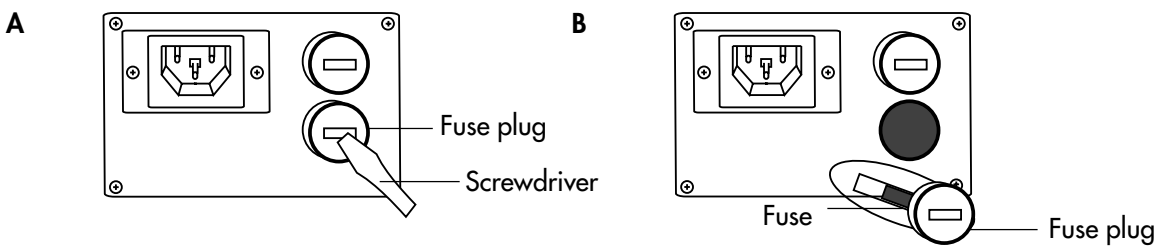
1. Disconnect the power cord from the back of the instrument. Move the power switch to the "0" (off) position.

2. Insert a small flat-head screwdriver into the slot in the center of the fuse plug (figure 9-1A), and gently turn. The plug will disengage. Pull the plug straight out to expose the fuse (figure 9-1B).

3. Remove both fuses and replace them with new ones (it is often impossible to determine visually which fuse is blown). You can also test the fuses with an ohmmeter to determine which is defective and replace just that one.

4. Gently press the fuse plug back into place. Turn and secure with the screwdriver. Reconnect the power cord.

Figure 9-1 A, How to pull out the fuse plug. B, Location of the fuses in the opened plug.



10 Troubleshooting

Sources of Problems

The Dyad cycler is designed to handle even the most stringent thermal-cycling requirements. A major strength of the Dyad cycler is its flexibility, which allows multifaceted and demanding cycling protocols to be implemented with the greatest of experimental and programming ease. However, occasional problems may still be encountered.

Problems can result in a number of ways. We group them as follows:

- System problems
- Problems in power up
- Problems in system performance
- Problems with Alpha units
- Problems due to environmental conditions, setup, and maintenance
- Problems related to protocols

When troubleshooting a difficulty, it is advisable to have the following pieces of information available, should it become necessary to contact MJ Research, Inc. for assistance:

- Serial and catalog numbers for the base as well as Alpha units
- Exact nature of the problem
- Frequency of the problem (i.e., is the problem repeatable)
- Steps already taken to troubleshoot the problem (i.e., controls performed)

System Problems

The Status window was reviewed in Chapters 5, 6, and 7, covering the creation, editing, and running of programs. For a review of the basic menus of this window, please review these chapters.

In the Status window, the <View> submenu allows the user to view the <Error Log>. Entries will be made to this log if a user terminates a program before its end, if the cycler terminates a program before end, or if the cycler encounters a serviceable issue before the termination of the program, but was able to complete the program. Problems and error messages can either be cycler or Alpha unit specific. In each case, a recommended action is listed and should be followed. Under no circumstances should a customer attempt service of a Dyad cycler or an Alpha unit, as this will result in a voided warranty and may not result in complete problem resolution. The only exception would be minor maintenance of the units, such as, removal of vent blockage or routine cleaning, as outlined in Chapter 9.

Table 10-1: Error Messages

The Error Codes listed in the table below correspond to the error codes returned in the Dyad cycler's <Error Log>. The Remote Error Codes listed correspond to the error codes returned when the Dyad cycler is operated in remote mode (see the <Remote> and <Auto Remote> sections in Chapter 8 for more information on remote mode).

Note: The Dyad system software is quite sensitive to block and heat-sink errors for these can affect accuracy and performance. When such error messages occur, try restarting the protocol. If the message fails to reappear, proceed as usual.

Remote Error Code	Error Code	Reason	Action
1	BO	This sample block reached a higher temperature than was expected.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
16	BSF-CI	An irregularity was detected in the block's center temperature sensor. The sensor will be checked automatically during and after the run, and you will be notified if any persistent problem is found.	No action required at this time.
19	BSF-LI	An irregularity was detected in the block's left temperature sensor. The sensor will be checked automatically during and after the run, and you will be notified if any persistent problem is found.	No action required at this time.
17	BSF-RI	An irregularity was detected in the sensitivity of the block's right temperature sensor. The sensor will be checked automatically during and after the run, and you will be notified if any persistent problem is found.	No action required at this time.
4	BSF-PT	An irregularity was detected in one or more of the block's temperature sensors. The sensor was checked and the results show an inaccuracy in block temperature measurement. Your program was automatically stopped.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
6	HLF	The heated lid is not pre-heating correctly. Your samples have remained at room temperature, and your program was never started.	Recover samples and call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).

Remote Error Code	Error Code	Reason	Action
8	**HSO**	The heat sink temperature is registering somewhat higher than normal. Power levels will be scaled back slightly until the heat sink temperatue returns to normal. Adequate airflow is necessary to prevent the heat sink from overheating.	Please observe the following guidelines: 1) place instruments at least 10cm apart, 2) avoid placing instruments in any location where hot air might enter the intake vents, 3) place instrument on a hard surface with no debris or paper underneath, 4) clean all air vents and Alpha unit fins of dust and debris, 5) avoid running the instrument in areas with ambient temperatures above 25°C. Continue to use the instrument. If problem reoccurs please call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
2	**HSO-PT**	The heat sink reached a temperature higher than normal, and the program was automatically stopped. Adequate airflow is necessary to prevent the heat sink from overheating.	Please observe the following guidelines: 1) place instruments at least 10cm apart, 2) avoid placing instruments in any location where hot air might enter the intake vents, 3) place instrument on a hard surface with no debris or paper underneath, 4) clean all air vents and Alpha unit fins of dust and debris, 5) avoid running the instrument in areas with ambient temperatures above 25°C. If the problem reoccurs please call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
5	**HS/PS-SF-PT**	Faults have been detected in sensors in the heat sink and power supply. To prevent overheating, the program has been automatically stopped.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
23,21, or 20	**HSSF**	An irregularity has been detected in a heat sink sensor. The sensor will be checked and you will be notified if any problem is found.	No action required at this time.

Remote Error Code	Error Code	Reason	Action
7	**IFF**	The internal fan is not providing adequate cooling for the instrument.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
22	**LSF**	An irregularity has been detected in a heated lid sensor. The heated lid has been temporarily turned off. The sensor will be checked and you will be notified if any problem is found.	No action required at this time.
None	**LRPI**	An imbalance has been detected in the sample block. There may be a problem with a Peltier module.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
None	**MC**	A memory fault has been found. Stored programs may be affected.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
None	**NMA**	All available memory has been filled.	Please delete unused programs, or call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
18	**PSF**	A possible fault was detected in the in-sample probe. The thermal control method has been switched to Calculated Mode. Results may have been affected.	Check probe and connections. If problem reoccurs please call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
9	**PSO-CAF**	The power supply temperature is somewhat higher than normal. The power supply sensor will be checked and you will be notified if any problem is found.	Please observe the following guidelines: 1) place instruments at least 10cm apart, 2) avoid placing instruments in any location where hot air might enter the intake vents, 3) place instrument on a hard surface with no debris or paper underneath, 4) clean all air vents and Alpha unit fins of dust and debris, 5) avoid running the instrument in areas with ambient temperatures above 25°C. Continue to use the instrument. If problem reoccurs please call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).

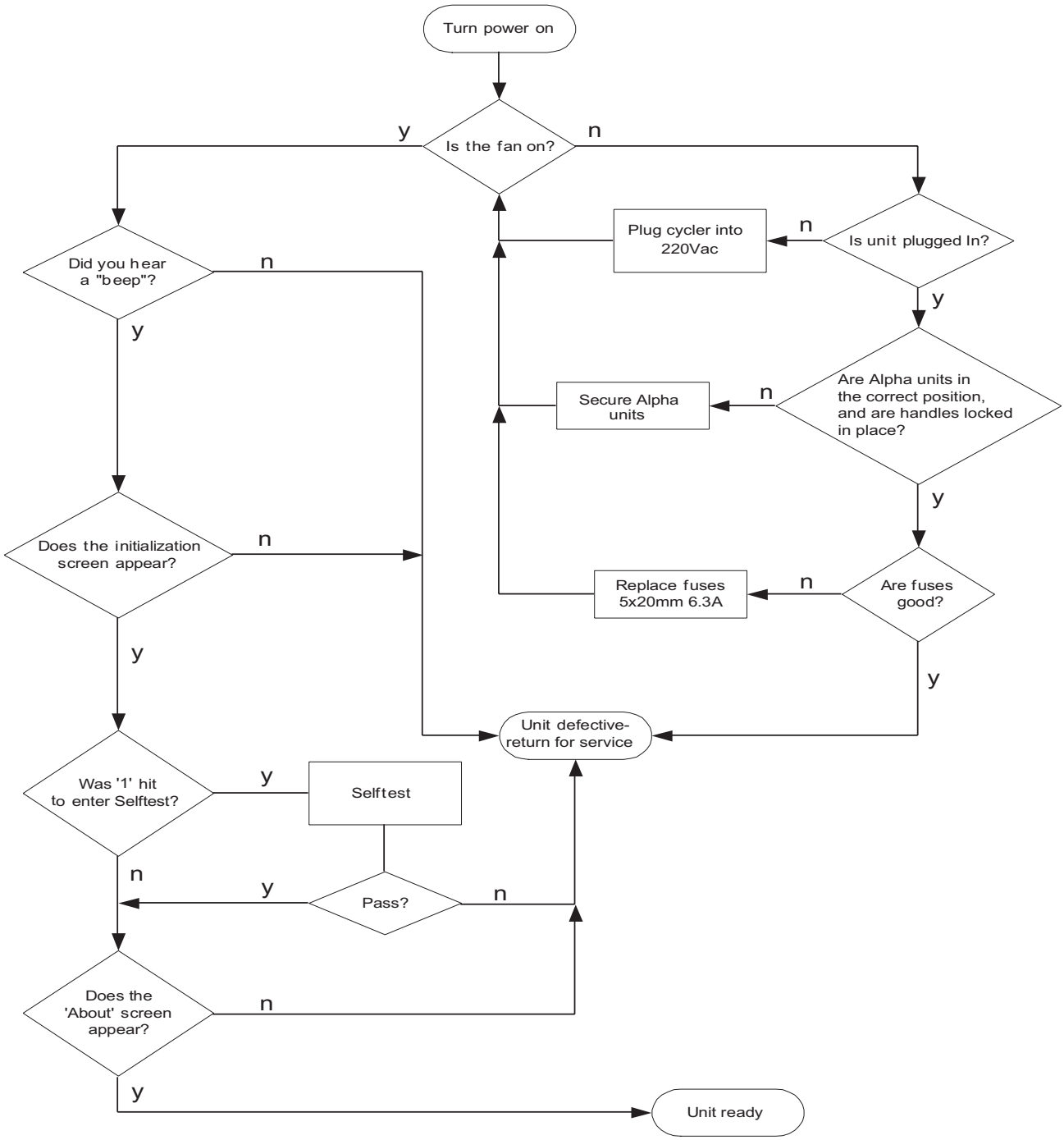
Remote Error Code	Error Code	Reason	Action
3	**PSO-PT**	The power supply temperature was higher than normal. To prevent instrument damage, the program was automatically stopped. Overheating may be caused by inadequate airflow or a problem with the power supply.	Please observe the following guidelines: 1) place instruments at least 10cm apart, 2) avoid placing instruments in any location where hot air might enter the intake vents, 3) place instrument on a hard surface with no debris or paper underneath, 4) clean all air vents and Alpha unit fins of dust and debris, 5) avoid running the instrument in areas with ambient temperatures above 25°C. Do not use the instrument until you call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
15	**PSSF**	An irregularity was detected in a power supply temperature sensor. The sensor will be checked and you will be notified if any problem is found.	No action required at this time.
12	**SBC-CAF**	The block took slightly longer than expected to achieve the programmed temperature. This condition may be caused by inadequate airflow, or a problem with the Alpha unit itself.	Please observe the following guidelines: 1) place instruments at least 10cm apart, 2) avoid placing instruments in any location where hot air might enter the intake vents, 3) place instrument on a hard surface with no debris or paper underneath, 4) clean all air vents and Alpha unit fins of dust and debris, 5) avoid running the instrument in areas with ambient temperatures above 25°C. Continue to use the instrument. If problem reoccurs call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
13	**SLC**	The heated lid took slightly longer than expected to reach its target temperature. There may be a problem with the lid sensor or lid heater.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).

Remote Error Code	Error Code	Reason	Action
None	**GPA**	A program containing a gradient step can only be run on a 96-well Alpha unit.	If you place a 96-well Alpha unit in this quadrant, you may run this gradient program.
None	**CCM**	The program entered may only run in Calculated or Block temperature control mode. Probe mode is unavailable for this type of program. For optimum results, the temperature control mode has been automatically switched to Calculated control.	No action required. Temperature mode has been automatically switched to Calculated control for this run only.
25	**GF**	The thermal gradient has not been achieved as quickly as expected. This may indicate a possible problem with the Alpha unit.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
10	**UF**	The instrument has detected unexpected temperature readings. This may indicate a problem with the Alpha unit.	Replace fuse on instrument and confirm that instrument is functional. If problem reoccurs please call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
26	**None**	A ground fault was detected in an Alpha unit.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).
27	**None**	A problem was detected in a Power Bonnet lid.	Call (888) MJ CYCLE (in the US or Canada), or your local distributor (outside the US or Canada).

Problems in Power-Up

Should a Dyad cycler not power up properly, as indicated in Chapter 4, please follow the steps outlined in the flowchart (figure 10-1) to determine the best course of action. In this case, a problem should be consistent and repeatable.

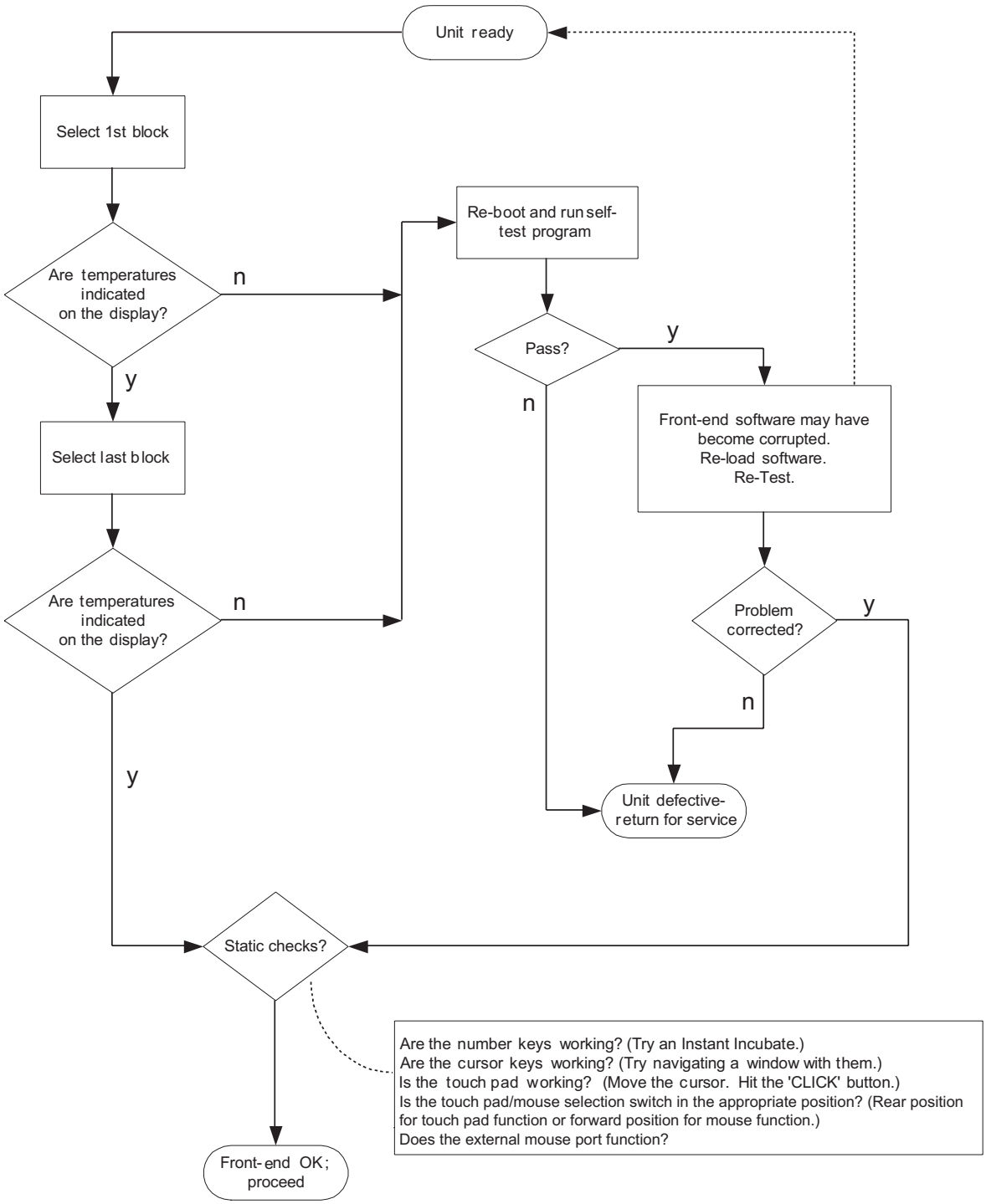
Figure 10-1: Power-up Troubleshooting Flowchart



Problems with System Performance

Should you encounter problems with menu navigation, front panel manipulation, or any performance aspect of a Dyad cycler that has successfully powered up, please follow the steps in this flowchart to determine the best course of action. Again, a problem should be a consistent, repeatable problem.

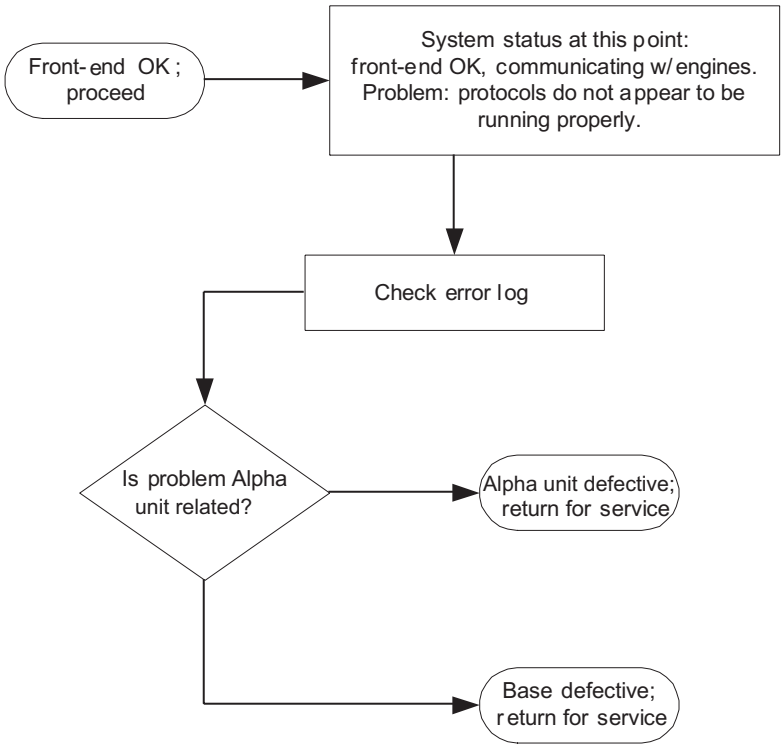
Figure 10-2: System Performance Troubleshooting Flowchart



Problems with an Alpha Unit

An Alpha unit is a distinct, separately engineered piece of equipment that is made to interface with all MJ Research DNA Engine line thermal cyclers. However, to rule out problems with any Alpha unit, please follow this recommended troubleshooting flowchart.

Figure 10-3: Alpha Unit Troubleshooting Flowchart



Problems Related to Protocols

The suggestions we make here are by no means exhaustive, but are intended as a starting point for further investigation. Should the Dyad system check out OK, we recommend positive controls for troubleshooting purposes.

Following is a general description of some common problems related to the protocols and reaction components in sequencing and thermal cycling applications. For a more detailed discussion of protocols and reactions, see *Current Protocols in Molecular Biology* (F. Ausubel et al., eds., John Wiley & Sons)

Problem	Cause	Action
No reaction products obtained.	Wrong protocol used.	Re-run reaction using correct protocol.
	Protocol contains a wrong value.	Use List utility to check protocol's temperature control method, temperatures, and times.
	Reaction component omitted from mixture.	Check reaction assembly protocol, ensuring that mixture contains appropriate components in correct concentrations.
	Denaturation temperature too low.	Use ≥92° C for denaturation. Only rarely are temps higher than 94°C required, however.
	Annealing temperature too high for primers.	Check for appropriate annealing temperatures of primers, using available computer programs or empirical testing.
	Wrong temperature control method used.	Use List utility to check temperature control method for protocol; change if needed.
	Probe failed, causing machine to run protocol under calculated control.	Check screen for probe failure error message. Probe may need servicing or replacing. Call MJ Research, Inc. or your local distributor.
	Probe not filled with correct amount of oil.	Fill probe tube with correct amount of oil (see p. 4-12).
	Reaction mix contains an inhibitor (e.g., heme from blood).	Test a complete reaction mix, minus sample, with a control template and primer set.
	Reaction vessels not making good thermal contact with sample block.	Use only high-quality tubes/plates that fit block snugly. Ensure that wells are free of foreign materials that would interfere with tube/plate seating.

Table 10-2: Protocol Difficulty

Error Message	Cause and Result	Action
Reaction is working but broad low molecular weight band is seen in gels.	"Primer-dimer" material often produces a broad band in the <100bp region of gels.	If obtaining appropriate reaction product/s, no need to change anything.
		Minimize "primer-dimer" production by designing primers with no 3' self-complementarity.
		Reoptimize magnesium concentration and annealing temperature to maximize desired product and minimize "primer-dimers."
Reaction working but unexpected extra products or smear is seen.	Nonspecific hybridization occurring during setup.	Program a hot start into the protocol.
	Reaction component concentration too high or too low.	Check concentratons of components. May need to reoptimize magnesium concentration.
	Annealing temperature too low.	Reoptimize annealing temperature.
	Protocol contains a wrong value.	Use List utility to check protocol's temperature control method, temperatures, and times.
	Template not of sufficient purity.	Check extraction and purification protocols. Add additional purification steps if necessary.
	Multiple templates or host DNA in sequencing reactions.	Check nucleic acid preparations by gel electrophoresis.

Problems due to Environmental Conditions, Setup, and Maintenance

Strict adherence to the installation, operation, and maintenance instructions provided in Chapters 3, 4, and 9 are tantamount to the continued trouble free operation of the Dyad cycler. Should it be determined that the source of a problem is due to incorrect operation or setup, consult Table 10-3 for a list of problems and suggested solutions.

Table 10-3: Environmental difficulties

Problem	Cause	Action
Frequent shutdowns due to overheating. Frequent "Slow Block Cycling," "HS Overheat," and "HS Overheating" error messages.	Machine is not receiving enough air.	Make sure air intake vents are not obstructed by dust, debris, or paper. Remove light collections of dust and debris with damp cloth. Vacuum out heavy collections. Remove any papers placed under the machine. Position machine at least 10cm from vertical surfaces.
	Air flowing into intake vents is not ≤ 31° C.	Check temperature of air entering air intake vents, following procedure on p. 3-4. If higher than 31° C, use Table 3-1 to troubleshoot and remove cause/s.
Dust and debris clogging up air intake vents.	Failure to regularly check for buildup.	Remove light collections with damp cloth. Vacuum out heavy collections.

11 Alpha Units and the Remote Alpha Dock System

Alpha™ Units Available from MJ Research, Inc.

Alpha™ unit, interchangeable sample-block/heat-pump assemblies are available in a palette of different configurations to accommodate a wide variety of thermal-cycling applications. All Alpha units are compatible with any of the cycler bases from the DNA Engine line including the DNA Engine™, Dyad™ and Tetrad™ bases. All Alpha units deliver the same thermal profiles with the same NIST-traceable accuracy no matter what instrument they are plugged into, and swapping an Alpha unit takes just ten seconds.

Available Alpha units include:

The "60" for 0.5ml Tubes (ALS-1260)
This block holds sixty 0.5ml microfuge tubes. Many researchers prefer this format because the tubes are easy to use, economical, and each is large enough to write on.

The "96" for 0.2ml Tubes or 96-well Plates (ALS-1296)
This sample format can hold either one 96-well microplate or 96 x 0.2ml tubes or strips of 0.2ml tubes. These V-well vessels are specifically designed for thermal cycling and they have become the industry standard. A thermal gradient ranging from 1°C up to 24°C can be programmed across this block allowing you to optimize reaction conditions in a single experiment.

The "384" for 384-well Plates (ALS-1238)
MJ Research, Inc. has worked with several genome centers to develop a high-density format for automated operation. The result is the 384-well Alpha unit, a true 4X version of the 96-well format. The wells have the same V-profile and are spaced on 4.5mm centers.

The Flat Block for Microarrays and Customized Attachments (ALS-1200, 384-well heated-lid; ALS-1201, 96-well heated lid; ALS-1203, no heated lid)
The Flat Block surface is ideal for microarrays and biochips, and it provides the flexibility to customize our industry-leading thermal cyclers for your specific needs.

The "30/30" Dual Block for 0.5ml Tubes (ALD-1233)
In many labs, multiple users compete for time on a thermal cycler, but rarely does a single user fill a 60-well block to capacity. At other times, an investigator may wish to run a single experiment with differing thermal parameters to optimize a protocol. For such circumstances, dual-block Alpha units are available. The blocks are independent; these hold 30 x 0.5ml tubes, and each has its own heated lid.

The "48/48" Dual Block for 0.2ml Tubes (ALD-1244)
An alternative dual design has two blocks that hold 48 x 0.2ml tubes or a 48-well microplate. Each independent block has an integral heated lid. The 0.2ml format works especially well in oil-free operation with small volumes ($\geq$5µl).

The "30/48" Dual Block for 0.5ml & 0.2ml Tubes (ALD-1234)

For those who wish to have available both the 0.5ml format and the 0.2ml format simultaneously, a combination Alpha unit is offered. This dual unit has two independent blocks with two different formats: one holds 30 x 0.5ml tubes and the other 48 x 0.2ml tubes or a 48-well plate. Each block has an integral heated lid. This Alpha unit has quickly become one of the most popular MJ Research units.

The "16/16" Twin Towers® Alpha unit for Glass Slides (ALD-0211, DNA Engine/Dyad cyclers; ALD-0212, Tetrad cycler)

This dual unit has two independent blocks, each of which can hold 16 slides in isothermal chambers that ramp at rates up to 1.2°C/second. Slides can easily be sealed with either Self-Seal™ reagent or Frame-Seal™ chambers, and the blocks can double as humidified chambers for hybridizations.

The "96" with a Power Bonnet™ motorized lid (ALP-1296)

This sample format can hold either one 96-well microplate or 96 x 0.2ml tubes or strips of 0.2ml tubes and features a Power Bonnet motorized lid for remote control of lid opening and closing.

The "384" with a Power Bonnet™ motorized lid (ALP-1238)

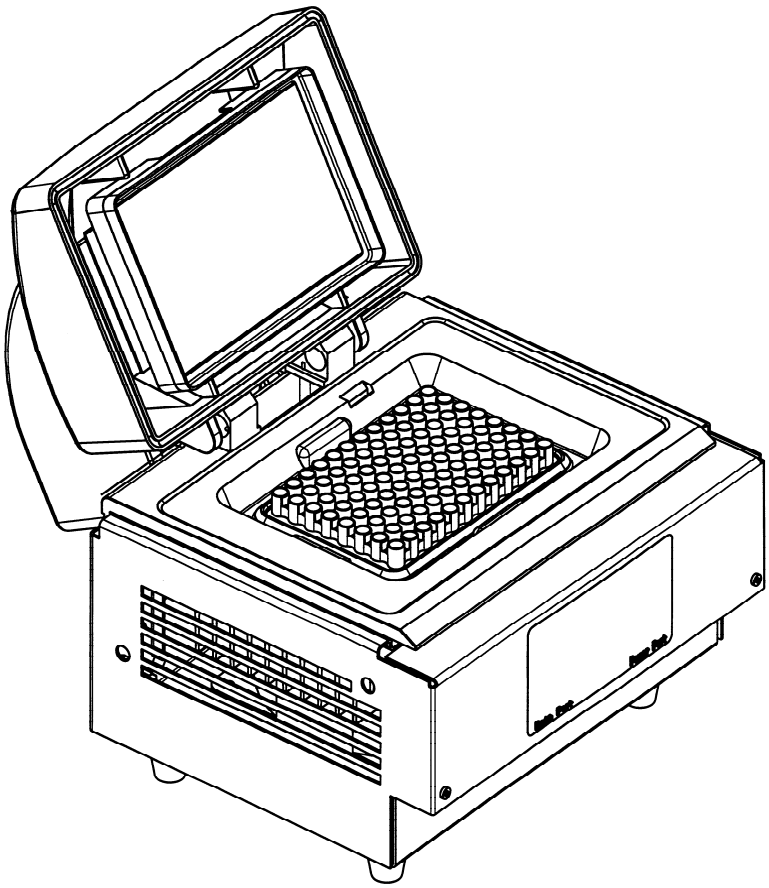
This sample format can hold one 384-well microplate and features a Power Bonnet motorized lid for remote control of lid opening and closing.

Contact MJ Research, Inc. at (888) 735-8437 for additional information on the Alpha units listed here, as well as updates on any new Alpha units available.

About the Remote Alpha Dock System

The Remote Alpha Dock™ system is designed to add flexibility to the installation and operation of the MJ Research PTC-220 DNA Engine Dyad cycler. The system allows Alpha units to be placed at a distance from the PTC-220 base, enabling more efficient use of space and facilitating robotic operation. The basic system, the RAD-200, comprises a dock connector, which mounts in the cycler base; and a Remote Alpha Dock base, into which the Alpha units are mounted. Additionally, the fan power supply along with the cables to run up to four Remote Alpha Dock fans from a single thermal cycler base must be purchased separately, as RPS-0200.

Figure 11-1 The Remote Alpha Dock base with Alpha unit mounted



Packing checklist

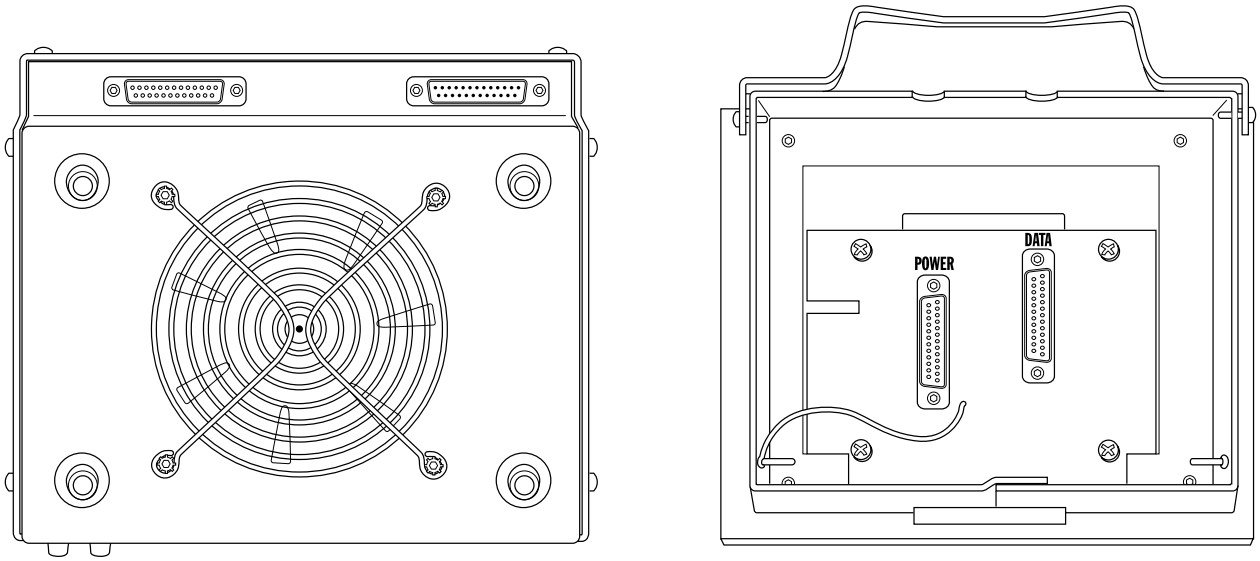
- One dock connector
- One Remote Alpha Dock chassis
- One multi-pin power cable
- One multi-pin data cable
- One fan power supply (RPS-0200)
- One wall-plug power cord (RPS-0200)
- Three round-jack power cords (RPS-0200)
- Product registration card (US and Canada only)
- Extended warranty application (US and Canada only)

Requirements

Environment

The Remote Alpha Dock system allows for custom installations. The following placement configurations are recommended by MJ Research, Inc.

Figure 11-2 Remote Alpha Dock base and dock connector, bottom view.

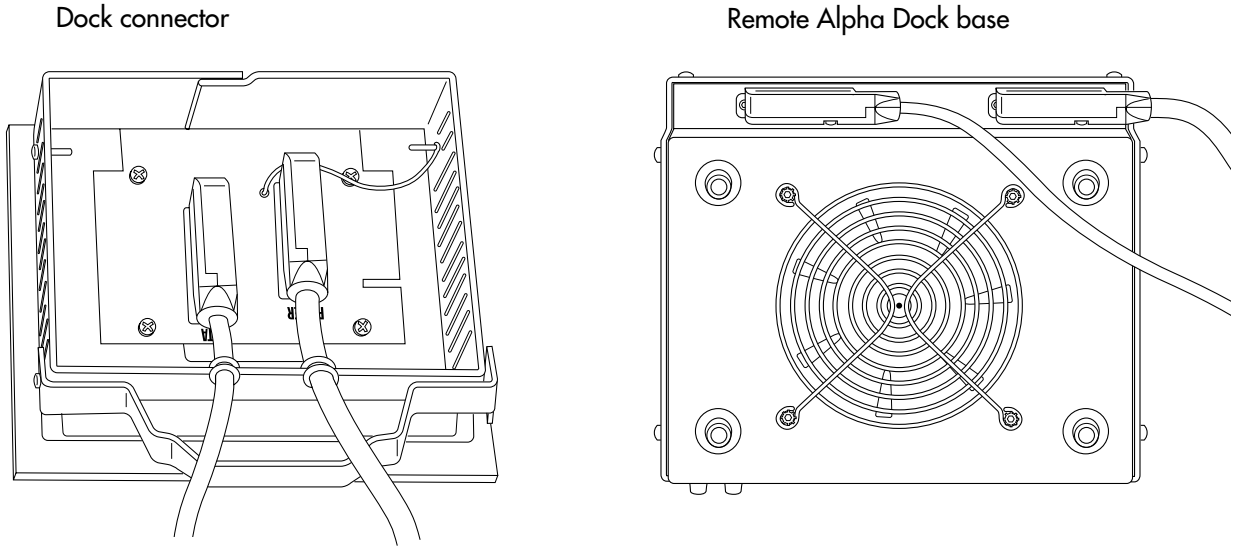


- Remote Alpha Dock bases with mounted Alpha units may be configured in any horizontal orientation or array as long as a minimum side clearance of 10 cm is maintained between the Remote Alpha Dock unit and any wall, bulkhead, or adjacent unit (this is identical to the PTC-220 base requirement). Requirements for motorized lid operation or for loading or unloading plates may dictate additional clearances.
- Remote Alpha Dock bases with mounted Alpha units may be stacked vertically as long as a minimum bottom clearance is maintained that would be no less than that resulting from the unit being placed on a solid horizontal platform. A minimum top clearance is also required to allow access to and operation of the Alpha unit lid.
- Remote Alpha Dock units can be flush-mounted (i.e., with the feet removed) to facilitate robotic operation, as long as the airway beneath the unit is equivalent to the airway the unit would have with the feet attached. Usually a hole will need to be cut to allow air to flow to the cooling fan. Figure 11-5 is a template for flush-mounting the Remote Alpha Dock unit.

Power Supply

- The Alpha unit mounted in each Remote Alpha Dock base is powered from the PTC-220 base.
- The Remote Alpha Dock unit's fan is powered externally, and a power supply is provided that requires power from 90-250 VAC and 47 to 63 Hz, with a grounded outlet.

Figure 11-3 Attachment of power and data cables



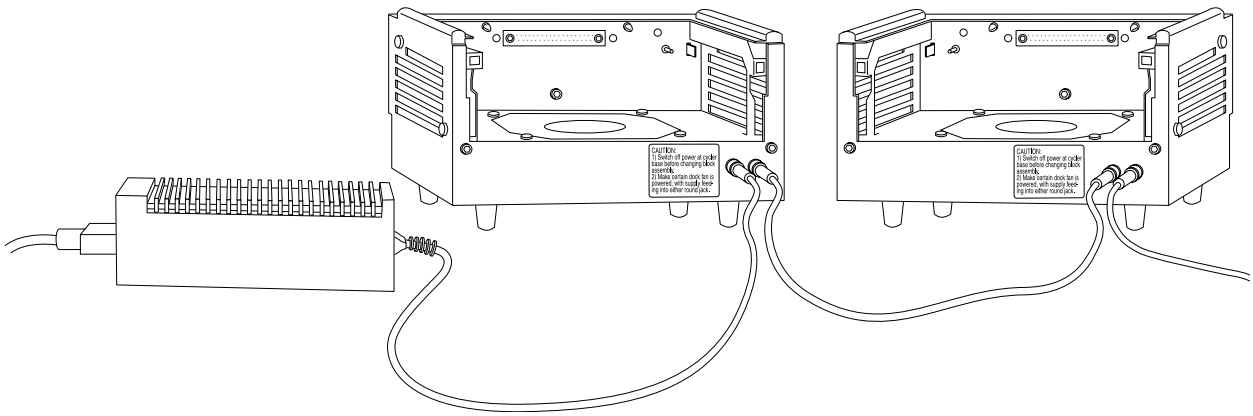
Air Supply

- Alpha units being operated in the remote configuration have no operating constraints that do not also apply to normal operations in the PTC-220.

Installation

- Turn the dock connector upside down, so that the green circuit board is visible. Check that the ground lead remains attached at both ends (fig. 11-1). Note the two female multi-pin sockets, one labeled "DATA," and the other "POWER."
- Connect the multi-pin power cable's male end to the female socket labeled "POWER" and slide the latch to lock the pins in place.
- Both of the data cable's multi-pin connectors are male: one is labeled "CONNECTOR DATA" and the other "DOCK DATA." Attach the "CONNECTOR DATA" end to the female connector labeled "DATA" on the circuit board and slide the latch to lock the pins in place.
- Press both cables firmly into the two strain relief holes on the Dock Connector's front side (fig. 11-2).
- Turn the Remote Dock upside down. You will see a male multi-pin connector labeled "Power Port" and a female multi-pin connector labeled "Data Port" (fig. 11-3).

Figure 11-4 Fan power supply connected in series



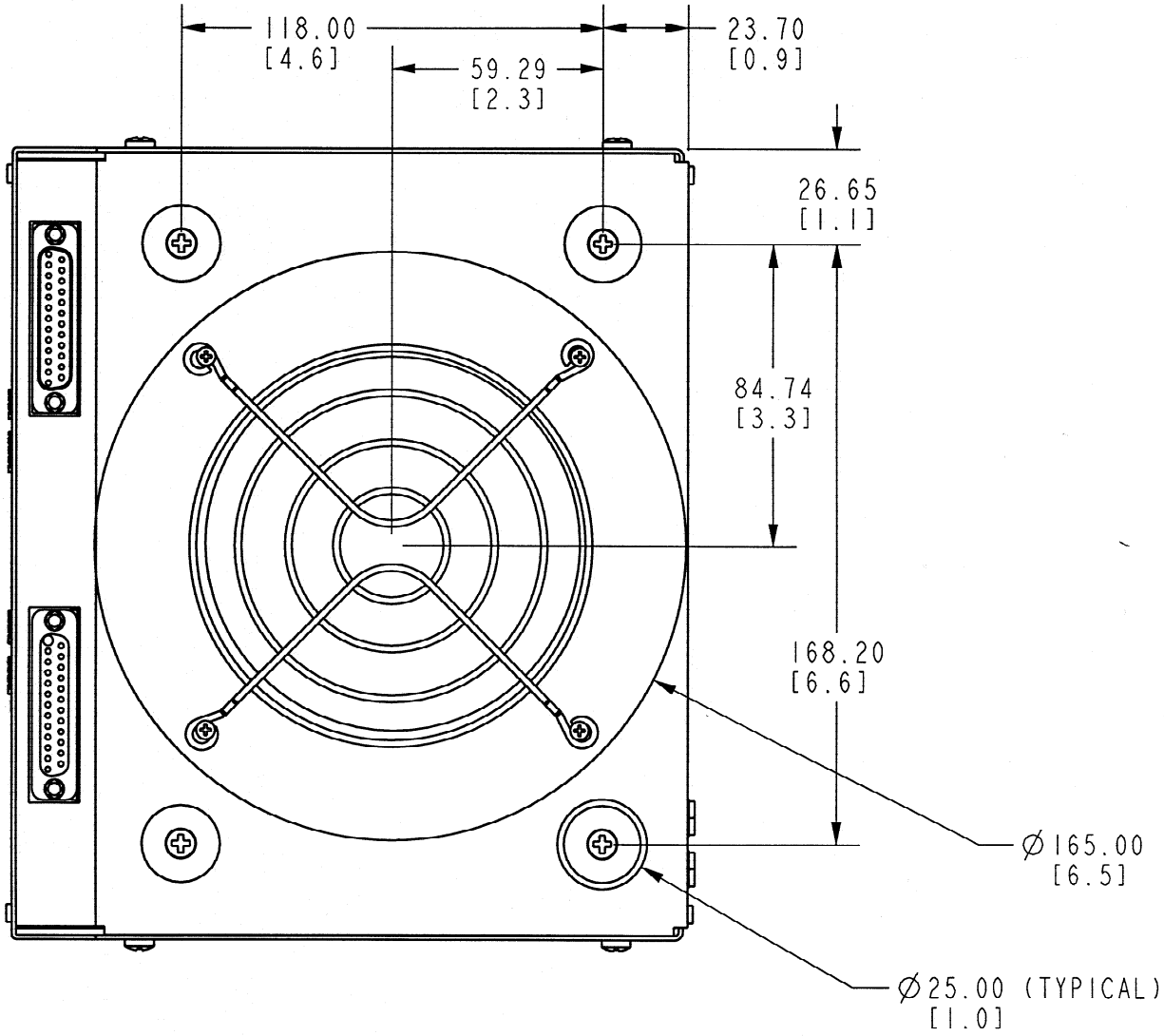
- Connect the multi-pin power cable's female end to the connector labeled "Power Port" and slide the latch to lock the pins in place.
- Attach the data cable's "DOCK DATA" end to the connector labeled "Data Port" and slide the latch to lock the pins in place.
- Turn both units back over.
- The dock connector mounts in the base in the same manner as a regular Alpha unit (see "Operating Alpha Units" in Chapter 4).
- Attach the wall-plug power cord to the fan power supply.
- Attach the fan power supply's round jack to either of the round connectors on the back of the Remote Dock (fig. 11-4).
- The fan power supply produces sufficient current such that up to three additional fans can be "daisy chained" to the initial Remote Alpha Dock unit via the round-jack power cords supplied. Attach one end of the cord to the initial unit's free round connector. Attach the other end to either of the round connectors on the back of the next Remote Alpha Dock unit in the series, and so on (fig. 11-4).
- When the Remote Alpha Dock system has been completely set up, connect the fan power supply's wall plug to a power source.

Operation

The Remote Alpha Dock system is transparent to the base unit; i.e., the dock connector allows the base to control the Alpha unit in the Remote Alpha Dock base as if it were in the standard configuration.

! **IMPORTANT:** Turn the base unit's power off when changing the type of sample block you are using. Turning the power off resets the base, allowing it to recognize the new block. If not reset, the base unit assumes that the previous type of block is installed, resulting in error messages and procedural faults.

Figure 11-5 Flush-mounting template



Appendix A

Safety Warnings and Guidelines

Warning: When removing an Alpha unit from a PTC-220 DNA Engine Dyad base, keep all fingers and foreign objects away from the Alpha unit bays. Keep all objects clear of the Alpha unit bays until the fan has come to rest.

Warning: Operating the PTC-220 DNA Engine Dyad cycler before reading this manual can constitute a personal injury hazard. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate these machines.

Warning: Do not open or attempt to repair the PTC-220 DNA Engine Dyad cycler base, any Alpha unit, or any accessory to the Dyad cycler. Doing so will void your warranties and can put you at risk for electrical shock. Return the PTC-220 DNA Engine Dyad cycler to the factory (US customers) or an authorized distributor (all other customers) if repairs are needed.

Warning: All Alpha unit blocks can become hot enough during the course of normal operation to cause burns or cause liquids to boil explosively. Wear safety goggles or other eye protection at all times during operation.

Warning: The PTC-220 DNA Engine Dyad cycler incorporates neutral fusing, which means that live power may still be available inside the machine even when a fuse has blown or been removed. Never open the PTC-220 DNA Engine Dyad cycler base; you could receive a serious electrical shock. Opening the base will also void your warranty.

Caution: Never remove an Alpha unit from the PTC-220 DNA Engine Dyad cycler with the power turned on and a program running. Doing so can cause electrical arcing that can melt the contacts in the connector joining the Alpha unit to the PTC-220 DNA Engine Dyad cycler.

Explanation of Symbols

Identifies components that pose a risk of personal injury or damage to the instrument if improperly handled.

Identifies components that pose a risk of electrical shock if improperly handled.

Identifies components that pose a risk of personal injury due to excessive heat if improperly handled.

Safe Use Guidelines

The PTC-220 DNA Engine Dyad cycler is designed to be safe to operate under the following conditions:

- Indoor use
- Altitude up to 3000m
- Ambient temperature 5°C–31°C
- Relative humidity 10–90%, noncondensing
- Transient overvoltage per Installation Category II, IEC 664
- Pollution degree 2, in accordance with IEC 664

Electromagnetic Interference

The PTC-220 DNA Engine Dyad cycler has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the US FCC Rules. These limits are designed to provide a reasonable protection against harmful interference when the equipment is operated in a commercial environment. These machines generate, use, and can radiate radiofrequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of these machines in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his or her own expense.

In addition, the PTC-220 DNA Engine Dyad thermal cycler designs have been tested and found to comply with the EMC standards for emissions and susceptibility established by the European Union at time of manufacture.

Further, the PTC-220 DNA Engine Dyad thermal cycler, does not exceed the Class A limits for radio noise emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

LE PRESENT APPAREIL NUMERIQUE N'EMET PAS DE BRUITS RADIOELECTRIQUES DEPASSANT LES LIMITES APPLICABLES AUX APPAREILS NUMERIQUES DE CLASS A PRESCRITES DANS LE REGLEMENT SUR LE BROUILLAGE RADIOELECTRIQUE EDICTE PAR LE MINISTERE DES COMMUNICATIONS DU CANADA.

FCC Warning

Changes or modifications to the the PTC-220 DNA Engine Dyad thermal cycler not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

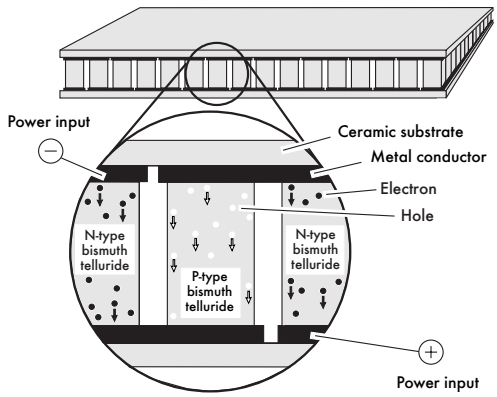
Appendix B

How a Peltier Heat Pump Works

The functional heart of every DNA Engine Dyad cycler is a high-performance Peltier-effect heat pump (also known as a "thermoelectric module"). The "MJ" module is a solid-state device manufactured to withstand the thermal stresses associated with rapidly cycling temperatures.

A thermoelectric module consists of numerous pairs of crystalline semiconductor blocks precisely sandwiched between two layers of ceramic substrate (figure A-1). The blocks are of two varieties: "N-type," which has a surplus of electrons in its crystalline structure, and "P-type," which has a deficit of electrons. The two types are positioned in alternating pairs within the innermost layer of the sandwich.

Figure A-1 A thermoelectric module.



The two types of blocks are wired together in alternating pairs. When electrical current is passed through the blocks, electrons in the N-type blocks and the "holes," or empty electron spaces, in the P-type blocks are excited at one conductor-semiconductor interface, which absorbs a small amount of heat. The electrons and holes flow through the crystalline blocks and return to a low-energy state at the other conductor-semiconductor interface, with the release of the previously absorbed heat. A thermal gradient of up to 70°C can be generated across the blocks in this manner.

The direction of heat pumping is reversed by reversing the polarity of current flow through the thermoelectric module, and the amount of heat pumped is changed by changing the amount of current passed. Both direction and amount of current flow are dictated by a microprocessor, allowing precise control of thermal cycling in the Alpha unit block.

Appendix C

Shipping Instructions for US Residents

Users residing in the United States should follow these instructions for shipping a machine to MJ Research for factory repair or an upgrade. Users outside of the United States should send machines to their distributor, in accordance with shipping instructions obtained from the distributor.

1. Call MJ Research (888-652-9253) to obtain a return materials authorization (RMA) number. Machines returned without an RMA will be refused by the Receiving Department.

2. Thoroughly clean the machine, removing excess oil and radioactive and other biohazardous substances. To protect the health of our employees, MJ Research will not repair or upgrade any machine that is excessively oily or that emits ionizing radiation upon arrival at our factory. **PLEASE ELIMINATE ALL BIOHAZARDS AND RADIATION!**

3. Pack the machine in its original packaging. If this has been misplaced or discarded, call MJ Research to request shipment of packaging materials. You can also request a loaner machine, which will be provided if available (a rental fee may apply). You can use the loaner's packaging to return the machine needing repair.

   Remove the Alpha unit from the DNA Engine Dyad base before shipping. All warranties are voided if a machine is shipped with an Alpha unit installed. If the Alpha unit also needs to be shipped, pack it in its original packaging materials.

4. Write the RMA number on the outside of the box.

5. Ship the machine (freight prepaid) to the following address. We recommend you purchase insurance from your shipper.

   Ship to: Repair Department
   MJ Research, Incorporated
   590 Lincoln Street
   Waltham, MA 02451

Appendix D

Warranties

U.S. & Canadian Limited Warranty, Standard

MJ Research, Incorporated warrants NEW MJ RESEARCH BRAND THERMAL CYCLERS (MODELS PTC-100, PTC-150, PTC-200, PTC-220 & PTC-225) against defects in material and workmanship for a period of two years from the date of purchase. If a defect is discovered, MJ Research will, at its option, repair, replace, or refund the purchase price of the THERMAL CYCLER at no charge to the customer, provided the product is returned to MJ Research within the warranty period. Refer to Appendix C for shipping instructions. In no event will MJ Research be responsible for damage resulting from accident, abuse, misuses, or inadequate packaging of returned goods and MJ Research disclaims all liability for consequential damages resulting from defects of any kind.

UNLESS OTHERWISE PROHIBITED BY LAW, ALL IMPLIED WARRANTIES INCLUDING MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE LIMITED IN DURATION TO TWO (2) YEARS FROM THE DATE OF ORIGINAL RETAIL PURCHASE OF THIS PRODUCT.

This warranty gives you specific legal rights. You may also have other rights that vary from state to state. Some states do not allow limitations on how long an implied warranty lasts, so the above limitation may not apply to you.

The warranty and remedies set forth above are exclusive and in lieu of all others, oral or written, expressed or implied. No MJ Research dealer, agent, or employee is authorized to make any modification, addition, or extension to this warranty, except in the form of the extended warranty outlined below.

MJ Research is not responsible for special, incidental, or consequential damages resulting from any breach of warranty, or under any other legal theory, including downtime, lost samples or experiments, lost reagents, lost profits, goodwill, damage to or replacement of equipment, property, and any costs of recovering or reproducing experimental results and data.

LIMITATIONS AND RESTRICTIONS: This warranty applies only to machines sold in the U.S.A. and Canada. Under no circumstance will MJ Research ship a repaired or replaced machine, or grant a refund of purchase price, to a user in a nation in which there was an authorized MJ Research distributor at the time of purchase. THIS WARRANTY IS NOT TRANSFERABLE FROM THE ORIGINAL PURCHASER TO A SUBSEQUENT OWNER. FURTHERMORE, THIS WARRANTY DOES NOT APPLY TO INSTRUMENTS USED OUTSIDE THE U.S.A. OR CANADA, EXCEPT WHEN EXPRESSLY AUTHORIZED IN WRITING BY MJ RESEARCH.

All provisions of this warranty are voided if the product is resold, repaired, or modified by anyone other than MJ Research or an authorized distributor.

U.S. & Canadian Extended Warranty, Optional

MJ Research, Incorporated will offer to each ORIGINAL PURCHASER of an MJ Research BRAND thermal cycler the opportunity to purchase an extension of the warranty coverage explained above for an additional two years. The coverage must be purchased through a purchase order received by MJ Research within 30 days of receipt of the offer of extended warranty or the offer to renew the extended warranty. These offers apply only to machines sold and used in the U.S.A. and Canada.

Index

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V

W

MJ Research, Inc.

The Originator of Peltier Thermal Cyclers

DECLARATION OF CONFORMITY

(for attachment of "CE" mark, as well as to document Canadian & US compliance)

MJ RESEARCH, INCORPORATED, manufacturer of the DNA Engine Dyad™ Peltier thermal cycler, Model PTC-220, hereby declares this equipment conforms to the following:

APPLICATION OF E.U. COUNCIL DIRECTIVES: 73/23/EEC, 89/336/EEC, 93/68/EEC

STANDARDS TO WHICH CONFORMITY IS DECLARED:

EU: **IEC 61010-1,** Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I, including Amendments 1 & 2

EU: **EN61326:1997, Annex B & A1:1998,** Electrical Equipment for Measurement, Control, and Laboratory Use, EMC Requirements, including Radiated & Conducted Emissions, and Immunity

CANADA: **CAN/CSA-C22.2 No. 1010.1-92 & No. 1010.1B-97,** Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part I, including Amndmnts 1& 2, CSA Cert. 1125786 (LR 97357-10)

US: **Underwriters Laboratories (UL) Std. No. 3101.1,** Electrical Equipment for Laboratory Use, Part I, CSA Int'l Certificate 1125786 (LR 97357-10)

US: **This device complies with Part 15 of the FCC Rules** ***(voluntarily)***

TYPE OF EQUIPMENT: European Union Class "A"; Electrical Equipment for Measurement, Control, and Laboratory Use

MODEL NUMBER: Model PTC-220 DNA Engine Dyad™ thermal cycler, rated 200-240VAC, 50-60Hz, 1.6 kW, cord connected. CSA International certifies as, "Equipment Class I, Pollution Degree 2, Installation Category II".

YEARS OF MANUFACTURE: July 2001 and onward

MJ RESEARCH INCORPORATED, manufacturer of the equipment described above, certifies this model of instrument has been tested and conforms to the applicable Directives & Standards of the European Union (EU), as well as those for Canadian and US compliance, as described above.

Test data to verify this conformity are available for inspection at MJ RESEARCH, INCORPORATED, both at the U.S. manufactory detailed below, as well as at the MJ Research European Representative Office at Kirke Værløsevej 16; 3500 Værløse, Denmark (+45) 44 35 05 40, fax (+45) 44 35 05 49.

5/1/02
Date signed

1
Copy number

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