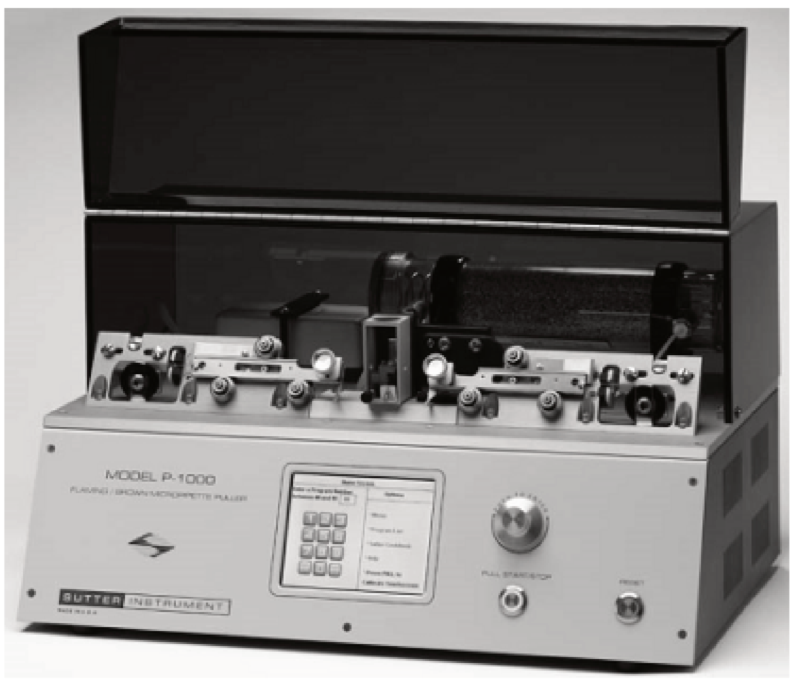
P-1000

Flaming/Brown™ Micropipette Puller System

Operation Manual

Rev. 2.30 (20140825)



SUTTER INSTRUMENT

One Digital Drive
Novato, CA 94949

Voice: 415-883-0128 Web: www.sutter.com
Fax: 415-883-0572 Email: info@sutter.com

DISCLAIMER

The pipette puller Model **P-1000** is designed for the specific use of creating micropipettes and no other use is recommended.

This instrument creates items that should only be used in a laboratory environment for use on animal tissues. It is not intended for use, nor should be used, in human experimentation, or applied to humans in any way. This is not a medical device.

Do not open or attempt to repair the instrument without expressed and explicit instructions from Sutter Instrument Company. Extreme heat and high voltages are present and could cause injury.

Do not allow unauthorized and or untrained operatives to use this device.

Any misuse will be the sole responsibility of the user/owner and Sutter Instruments assumes no implied or inferred liability for direct or consequential damages from this instrument if it is operated or used in any way other than for which it is designed.

SAFETY WARNINGS AND PRECAUTIONS

Electrical

- Operate the P-1000 using 110-120 V AC, 60 Hz, or 220-240 V AC., 50 Hz line voltage. This instrument is designed for connection to a standard laboratory power outlet (Overvoltage Category II), and because it is a microprocessor--controlled device, it should be accorded the same system wiring precautions as any 'computer type' system. A surge protector and power regulator are recommended.
- Fuse Replacement: Replace only with the same type and rating:

  Type: 5 x 20 mm glass tube, Medium Time Delay (IEC 60127-2, Sheet III) or Time Lag, RoHS compliant.

  Rating: T4A 250V (Time Delay, 4 Amps, 250 Volts)

  Examples: Bussmann S506-4-R or Littelfuse 218 004.P (or 218 004.HXP)

  A spare fuse is located in the power input module. Please refer to the fuse-replacement appendix for more details on fuse ratings and for instructions on how to change the fuse.

Avoiding Electrical Shock and Fire-related Injury

- Always use the grounded power supply cord set provided to connect the system to a grounded outlet (3-prong). This is required to protect you from injury in the event that an electrical hazard occurs.
- Do not disassemble the system. Refer servicing to qualified personnel.
- To prevent fire or shock hazard do not expose the unit to rain or moisture.

Back Injury Prevention

To avoid injuring your back or limbs it is recommended that you do not attempt to lift this instrument by yourself. The P-1000 Micropipette Puller weighs in excess of 16 kg (over 35 lb) and should be moved by TWO (2) people.

Operational

Failure to comply with any of the following precautions may damage this device.

- Operate the P-1000 using 110-220V AC., 50-60Hz-line voltage.
- The P-1000 is designed for operation in a laboratory environment (Pollution Degree II) and at temperatures between 5°C - 40°C.
- This unit is not designed for operation, nor has it been tested for safety, at altitudes above 2000 meters (6562 feet).
- This unit was designed to operate at maximum relative humidity of 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C.
- Operate only in a location where there is a free flow of fresh air on all sides. NEVER ALLOW THE FREE FLOW OF AIR TO BE RESTRICTED.
- To avoid burns do not touch the heating filament, the brass clamps holding the filament, or the heated ends of glass pipettes that have been pulled.
- Only use Sutter Instrument Company replacement heating filaments.

Handling Micropipettes

Failure to comply with any of the following precautions may result in injury to the users of this device as well as those working in the general area near the device.

- The micropipettes created using this instrument are very sharp and relatively fragile. Contact with the pulled micropipette tips, therefore, should be avoided to prevent accidentally impaling yourself.
- Always dispose of micropipettes by placing them into a well-marked, spill-proof “sharps” container.
- Use only with capillary glass (tubing) recommended by Sutter Instrument Company in the following section of this manual (1.2.1).

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

1. GENERAL INFORMATION

1.1 Introduction

The P-1000 can fabricate pipettes for use in intracellular recording, patch clamping, transferring (ICSI, ES Cells), microinjection, aspiration, and microperfusion. Realizing the full potential of this instrument is dependent on a complete understanding of the way it implements the pulling process. To this end, we urge that this manual be read in its entirety. To aid in understanding the function of the instrument, sample programs are already loaded in memory (Programs 0 through 5, as discussed in subsequent material).

The Model P-1000 Flaming/Brown Micropipette Puller combines a proven pulling technology with programmability to produce a very versatile instrument. The pulling mechanism is derived from the Flaming/Brown series of pullers, which have demonstrated the ability to pull a complete range of pipette profiles. Added to this mechanism is the ability to program different pulling sequences; thus, allowing ease of use for pulling a variety of pipette types on one device.

The P-1000 is a 'velocity sensing' puller. This patented feature allows the puller to indirectly sense the viscosity of the glass, giving the P-1000 the ability to pull pipettes from all glasses except quartz. Even difficult to pull formulations, such as aluminosilicate glasses, are handled with relative ease.

Throughout this manual reference will be made to the size of the glass tubing used to pull micropipettes. The convention used here for describing the outside diameter (O.D.) and inside diameter (I.D.) is as follows: O.D. x I.D. These dimensions will always be given in millimeters (mm). See our catalog or visit www.sutter.com to find a wide selection of glass capillaries available for purchase.

1.2 Glass Capillary & Heating Filament Specifications

1.2.1 Glass Capillary

The P-1000 micropipette puller is designed for use with aluminosilicate, borosilicate or other lower melting-point glass tubing or rod ranging from 0.6 to 3.0 mm in diameter. This instrument does not pull quartz glass. Examples of the specific types and sizes of glass that can be used with the P-1000 are listed in the Sutter Instrument Company catalogue that was included with this instrument or can be viewed on Sutter Instrument's web site at www.sutter.com. Any glass with comparable technical specifications can be used with the P-1000.

1.2.2 Heating Filament

The type and size of glass that you choose may require a Heating Filament other than the one installed in your puller at the factory. Please refer to the Heating Filament section of this manual to determine the appropriate style and size of filament necessary for pulling the specific glass you would like to use. This instrument is designed to accommodate any of the Sutter Instrument Trough-type or Box-type filaments that are shown in the Sutter Instrument catalogue. This selection of replacement filaments can also be viewed on Sutter Instrument's web site at www.sutter.com.

NOTE: USE ONLY SUTTER INSTRUMENT-SUPPLIED REPLACEMENT HEATING FILAMENTS IN THIS INSTRUMENT!

Systematic instructions for replacing the heating filament can be found in the Maintenance section of this manual and in the Help Menu of the P-1000.

1.3 Technical Support

Unlimited technical support is provided by Sutter Instrument Company at no charge to our customers. Our technical support staff is available between the hours of 8:00 AM and 5:00 PM (Pacific Standard Time) at (415) 883-0128. You may also Email your queries to info@sutter.com.

1.4 System Description – Front Panel

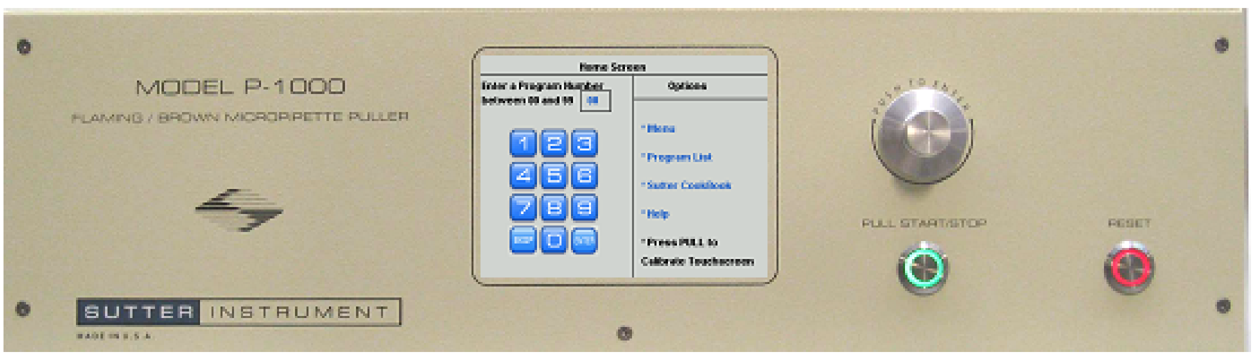


Figure 1-1. P-1000 front panel.

Color Touchscreen

Provides a user interface with touch sensitive buttons and text descriptions. All touch-sensitive items are blue, including text in blue.

A keypad or keyboard is displayed where needed

Reset

The Reset button is used to perform an emergency reset of the P-1000's microprocessor.

Pull Start/Stop

Starts and stops the pull cycle

Rotary Dial

Used as an alternative to the displayed keypad for numeric entry. Turn right (clockwise) to increase the currently displayed value, left (counterclockwise) to decrease, and press in to enter the currently displayed value.

Rotating the dial also allows scrolling up or down a list of choices.

1.5 System Description – Mechanical (Puller Anatomy)

1.5.1 Some Basic Information

This section presents a basic mechanical description of the P-1000, with particular emphasis on terminology. Knowing the names of the various parts greatly facilitates communication between the investigators and the manufacturer when discussing adjustments or service problems. In addition, various controls and adjustments on the top of the instrument are located and described. Those adjustments, which are considered part of maintenance procedures, are dealt with in the Maintenance Section of this manual.

1.5.2 Air Cooling System

The Model P-1000 supplies a blast of air to cool the filament area after the heating segment of a pull cycle. The components of the air-cooling system are shown below.

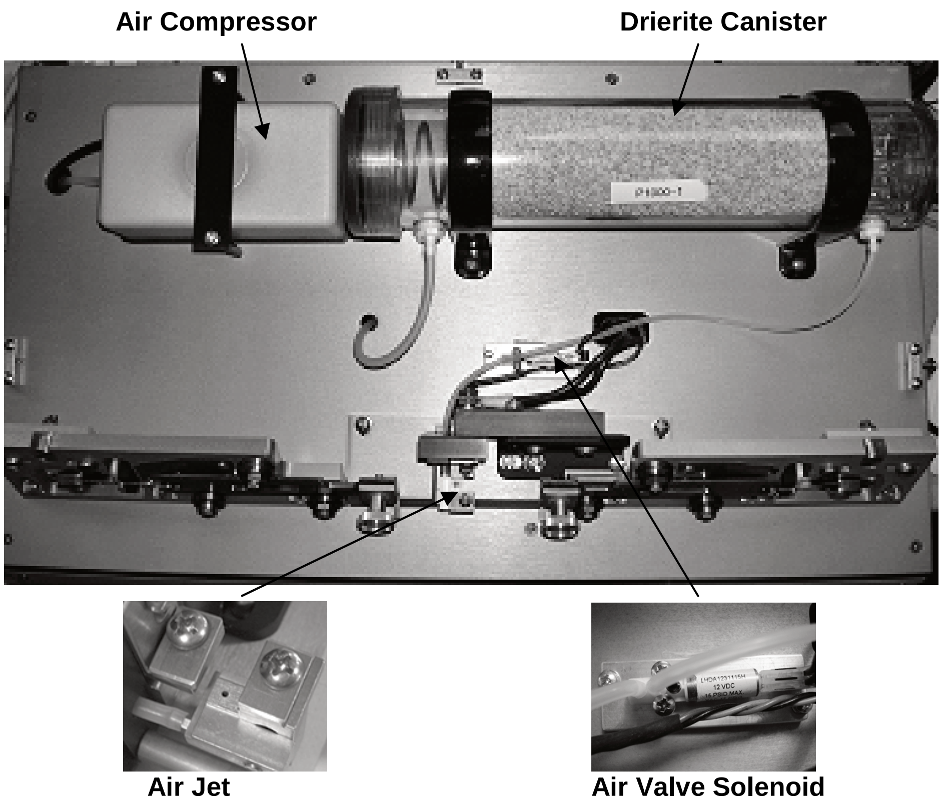


Figure 1-2. P-1000 base plate with components detail.

Air Compressor — The air compressor (or pump) creates the air pressure used to cool the filament and glass during the pull cycle.

Air Jet — Directs the cooling air to the filament. The air jet should be positioned 2 to 3 millimeters below the filament. The screw that secures the air jet to the filament block can be loosened allowing the jet to move up and down.

Air Valve Solenoid Regulates the flow of cooling air to the filament and glass.

Drierite Canister A canister filled with Drierite used to remove moisture from the air flowing between the pump and the air solenoid. The dissected air is used to purge the Humidity Control Chamber before and after a pull, allowing the flow of dry air to cool the filament and glass during the pull cycle.

1.5.3 Heating Assembly

The **Heating Assembly** comprises the **Filament, Filament Block Assembly** and the **Humidity Control Chamber**. The Filament Block Assembly and the Humidity Control Chamber are discussed below. Filaments are discussed in a separate section.

Humidity Control Chamber (Figure 1-3) The chamber encloses the filament block assembly to provide a controlled environment in which to pull the glass. Access holes in the side of the chamber allow the glass to be loaded into position. The chamber must be removed to access the heating filament. To remove, unscrew the thumbscrew on the front plate of the chamber, remove the front plate, and then pull the chamber towards you.

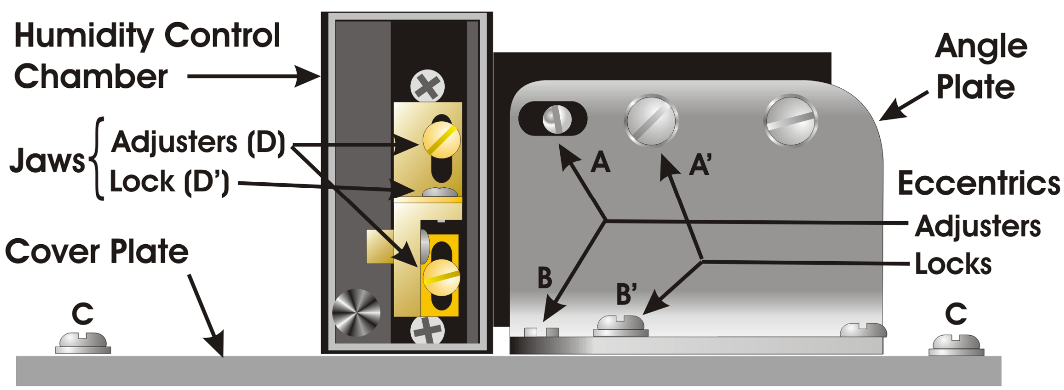


Figure 1-3. Filament block assembly.

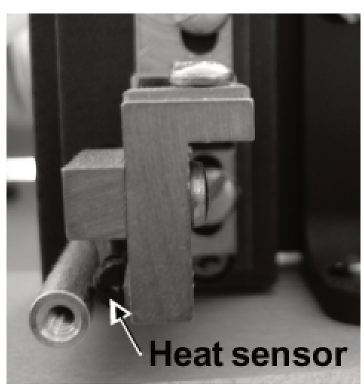


Figure 1-4. Heat sensor location on filament block assembly.

Filament Block Assembly (Figure 1-3)

The filament block assembly is made up of several pieces of hard black nylon. Wires supplying current to the filament are attached to threaded 'posts'. This current is carried to the filament via the upper and lower **Brass Jaws**. Note that these jaws may be moved up and down by loosening the screws (**D**) that secure them to the front of the filament block assembly. When changing the filament type from trough to box (or vice versa), the jaws must be moved up or down so that the filament is positioned at the correct level relative to the glass. If the jaws are repositioned and/or the filament type has been changed, make sure that the securing screws of the jaws and filament clamp are again tightened. Failure to tighten these screws can result in, poor current flow for scorching and insufficient heat to melt the glass. Please refer to **NOTE: See Sutter Instrument YouTube video "Installing a Filament** (https://www.youtube.com/watch?v=cCsJsIZlzLw)**.**

Filament Replacement in the Maintenance chapter for additional instructions.

Eccentrics and Angle Plate (Figure 1-3)

The Angle Plate secures the Filament Block Assembly to the Cover Plate; it contains two eccentric adjustments. The two chrome-plated screws **A** and **B** are the eccentrics, and **A'** and **B'** are the corresponding locking screws. By turning the eccentrics with a screwdriver the Filament Block Assembly can be moved up and down (**A**) or forward and back (**B**) to adjust the position of the filament. Loosen the locking screw associated with each 'eccentric screw' before turning, and tighten after completing the adjustment. Note: Changing the eccentrics should be made only for **fine/small adjustments**.

Cover Plate (Figure 1-3)

The cover plate conceals the entry of the Pulling Cables into the Base of the instrument. It is attached to the top by two screws, in slots, at points labeled C. Loosening these screws allows the Filament Block/Angle Plate assembly to move forward and back over large distances.

NOTE: The movements of the Cover Plate and the Jaws constitute the 'coarse adjustments' of filament position, while the eccentric screws allow 'fine adjustments'.

1.5.4 Upper Pulley Assembly

This assembly guides the **Pulling Cables** (**T in Figure 1-5**) from the **Puller Bars** (**G in Figure 1-5**) to the centrally located (and concealed) **Lower Cable Pulley Assembly**. Note that the **Upper Cable Pulley Assembly** is attached to its panel by two screws, in slots (**J' in Figure 1-5**), and contains a large eccentric adjustment screw (**J in Figure 1-5**). This eccentric screw is used to adjust cable 'tension'. Its use is covered in the Maintenance Section, and changes to the settings should not be performed without the supervision of Sutter Instrument Technical Support.

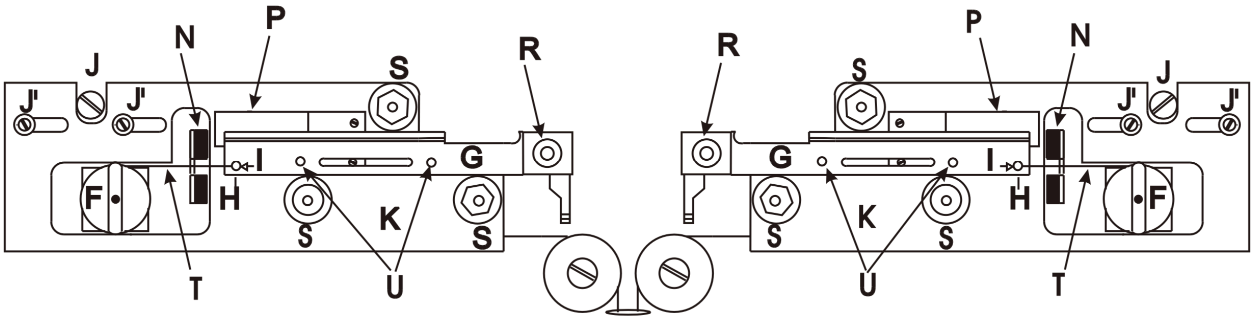


Figure 1-5. Upper cable pulley assembly.

Panels, Left And Right (K in Figure 1-5)	The panels are the angled surfaces that provide mountings for the Puller Bars and their Bearings, the Spring Stops, the Bumpers, and the Upper Cable Pulley Assemblies. Except for minor differences in shape, the left and right Panels are mirror images of each other. Note the three socket-head cap screws that attach each Panel to the base plate top. These screws are used to align the Puller Bars. Their adjustment, if necessary, is covered in the Maintenance Section. Contact Sutter Instrument Technical Support for more instructions on how the panels are aligned.
Bumpers (N in Figure 1-5)	The Bumper stops the motion of its associated Puller Bar. Each Bumper also prevents impact forces from breaking pipettes.
Spring Stops (P in Figure 1-5)	The Spring Stops are one-way catches that catch the Puller Bars as they rebound off the Bumpers so as to prevent pipette tip collision.
Puller Bars (G in Figure 1-5)	This assembly consists of the puller bar, threaded post, electrode clamp knob, and cable retaining screw. The cable retaining screw (H) holds the cable in a shallow groove (I) at the end of the puller bar, and forms the ‘resistance’ against which the cable ends pull. The puller bar is made of mild steel and coated with a controlled thickness of hard chrome. Glass is loaded into the groove near the tip of the puller bar and is held in position by tightening down the clamping knob (R).
V- Bearings (S in Figure 1-5)	These bearings are the guides for the Puller Bar motion. They are made of stainless steel and must NEVER be oiled (see Maintenance Section). Note that these bearings are mounted on stainless steel bushings, one of which is round with the other two being hexagonal. The hexagonal (eccentric) bushings are used to adjust position and ease of travel of the PULLER BARS (see Maintenance Section). Do not adjust the eccentrics without additional instruction.

Pull Cable (T in Figure 1-5)	This cable transmits the pulling force of the solenoid to the Puller Bars via the Upper (F) and Lower Pulley Assemblies. It is made of flexible metal with a nylon coating. Never pinch or distort the cable. The cable is terminated with crimped-on clamps or 'swages' at the back-end of each Puller Bar.
Glass Stop Mounting (U in Error! Reference source not found.)	Holes for mounting the glass stop. Either the left side or the right may be used.

1.5.5 Cabinet

Baseplate	The top thick metal plate on which the mechanical assemblies are mounted.
Base	The Base includes the cabinet to which the top Baseplate is mounted as well as the transformers and the circuit board contained within.

1.5.6 Electronics

The P-1000 micropipette puller is controlled by a dsPIC microprocessor. The HEAT power supply is a precision constant-current switching unit. The PULL supply is a constant-current DC power supply. The velocity trip point is set by a D-A converter. The output of the velocity transducer is compared to the output of the velocity D-A to determine when the trip velocity is reached.

(This page intentionally blank.)

2. INSTALLATION

2.1 Unpacking

Make certain that you have received all of the following items in the P-1000 shipping box:

- P-1000 micropipette puller*
- Power cord
- Sample box containing the following types of glass:
  BF100-50-10 (Microinjection)
  BF150-86-10 (Thick-Walled Patch (dissocated or cultured cells))
  BF150-110-10 (Thin-Walled Patch (slice or whole tissue))
- Four spare heating filaments (FB255B, FB330B, FB245B, and FT330B)
- Warranty registration
- Sutter Instrument Product Catalog
- P-1000 & P-97 Pipette Cookbook
- Glass Stop
- BX-10 Pipette Storage Box
- Parameter Guide "cheat sheet" card

The Model P-1000 micropipette puller is shipped to you in a custom box with foam inserts. Please save shipping materials for future use. Should it ever be necessary to ship the puller to another location, the same packaging should be used to prevent damage to the instrument. Additional packing material may be purchased from Sutter Instrument Company.

IMPORTANT: Improper packaging is a form of abuse and, as such, can be responsible for voiding the warranty where shipping damage is sustained because of such packing.

2.2 Setting Up

2.2.1 Line Power (Mains)

The P-1000 is designed to operate with a mains power of 110–220 V AC, at 50-60 Hz. The power cord provided with the P-1000 connects the grounded mains power outlet to the Power Entry Module located on the back of the unit (see diagram below). This Module also includes the Line Fuse.

* Unless specified otherwise, the P-1000 is shipped equipped with, and pre-programmed for, a 2.5mm box heating filament.

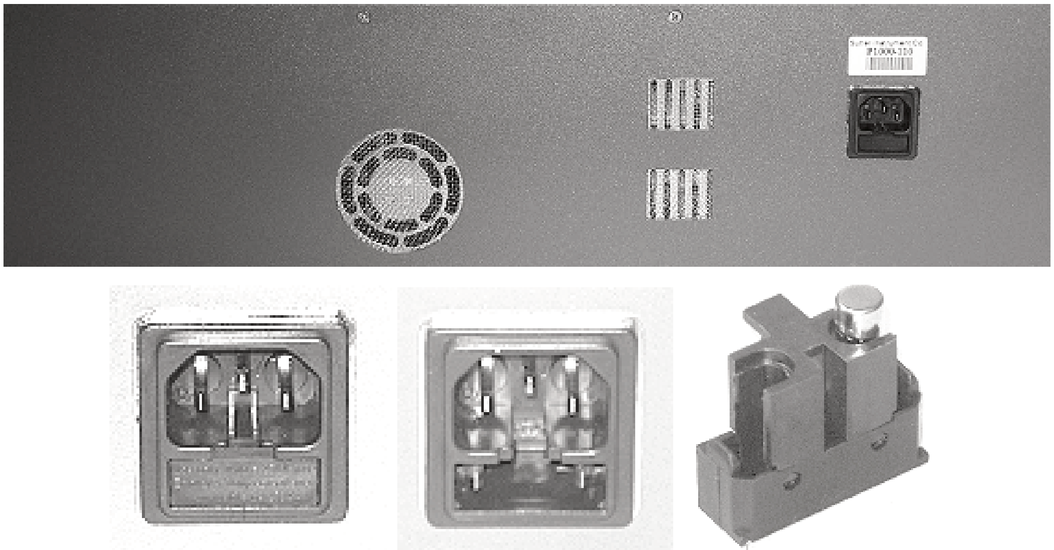


Figure 2-1. P-1000 Cabinet (rear view), detailing power entry module, fuse-holder, fuse, and spare fuse holder.

Make certain that the Power Switch located on the left end of the P-1000 cabinet is OFF (**O**).

Figure 2-2. P-1000 Cabinet (end view, left), showing power switch.

Plug the power cord provided with the P-1000 into the Line Input socket on the Power Entry Module and then to a power source.

3. OPERATING INSTRUCTIONS

3.1 First Time Use

While we realize that most new users of the P-1000 are anxious to start pulling useable pipettes right away, we cannot over-state the importance of taking a few moments to review the manual in order to understand how the puller works. Many a heating filament has been destroyed with first use due to not taking the time to understand the relationship between the programmable heat settings and the filament installed in the puller. If you absolutely must use the puller before reading through the manual, the following instructions are provided to help you get going and keep you from vaporizing your heating filament.

3.1.1 Quick Start Instructions

1. Remove the rubber bands from the P-1000 puller bar knobs.
2. Plug in the puller to the power AC mains.
3. Turn the puller ON – the power switch is on the left-side panel.
4. The Startup Screen will be displayed for 5 seconds while the puller systems are checked.

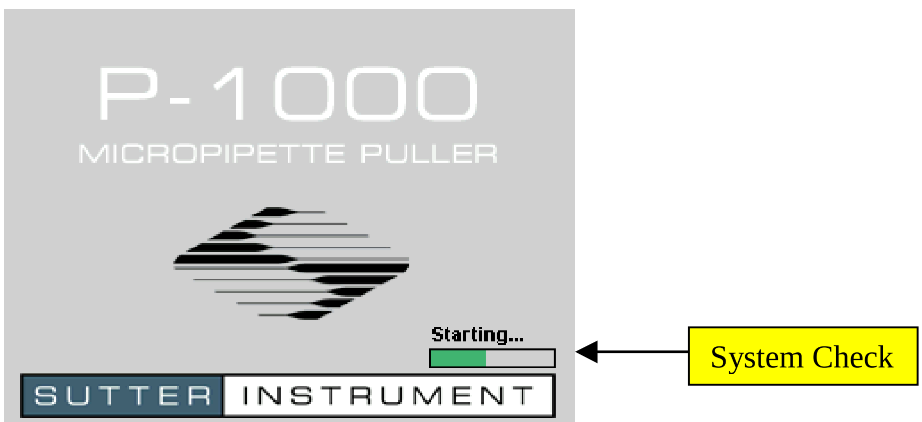


Figure 3-1. Startup screen.

5. The Home Screen will appear.

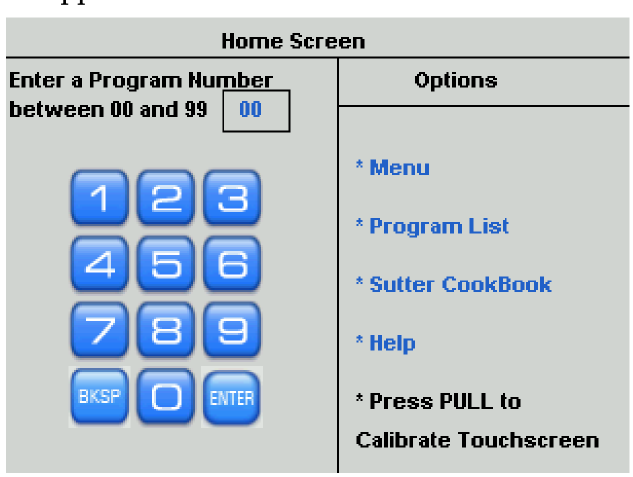


Figure 3-2. Home screen.

6. Use knob or keypad to select Program # [3]. Press <ENTER> or push IN the knob to enter Program 3.

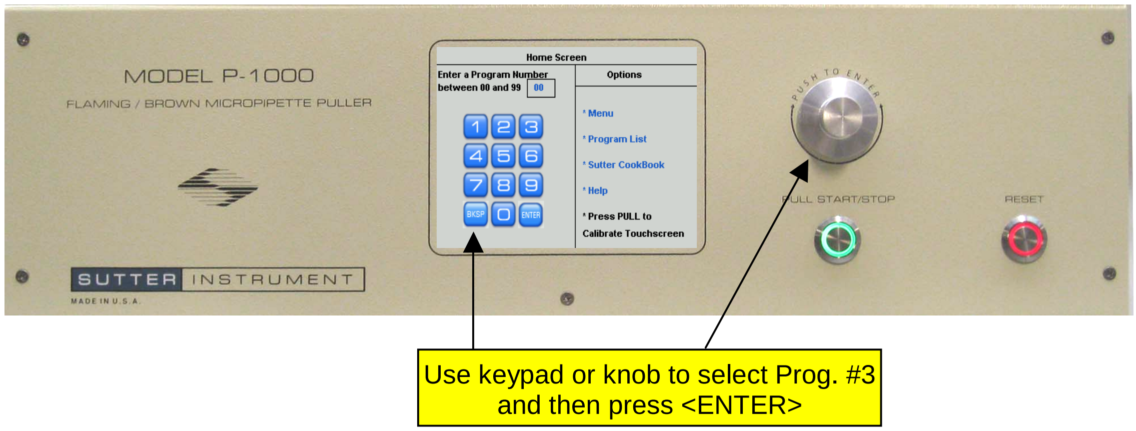


Figure 3-3. Using the knob or keypad to select a program.

7. Program 3 will now be displayed.

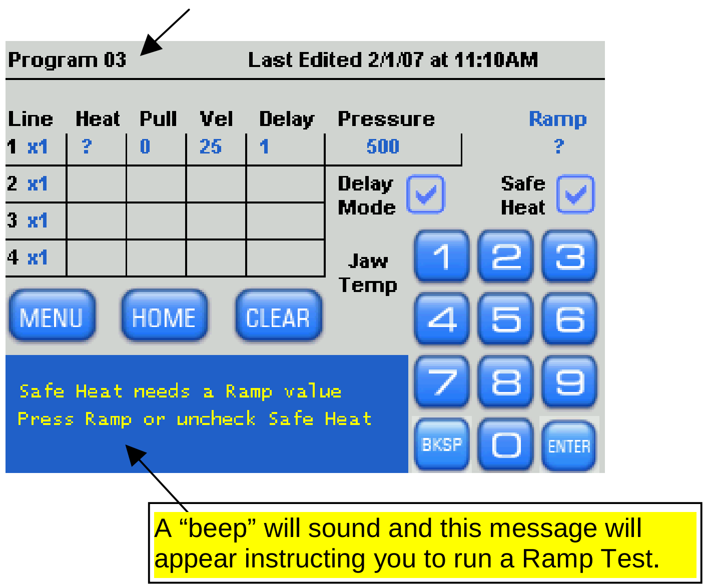


Figure 3-4. Instruction to run ramp test.

8. Press "Ramp" on the touch-screen display to run a Ramp Test.

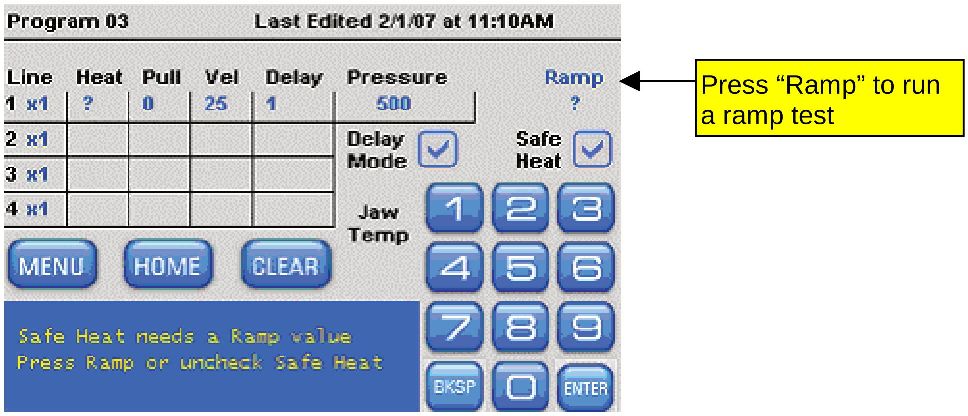


Figure 3-5. Running the ramp test.

9. Now you will see the Ramp Test Screen.

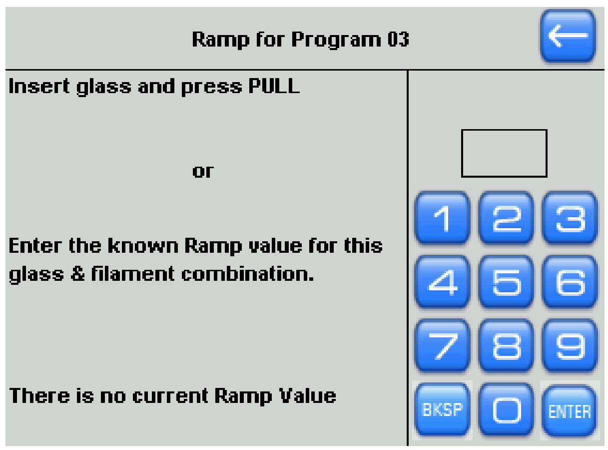


Figure 3-6. Ramp Test screen.

The ramp test helps to establish safe heat settings. The ramp value is unknown for the 2.5 x 2.5 box filament (FB255B) filament in your puller and the 1.5 x 0.86 glass you will be using, so you will need to run a Ramp test.

10. Install a piece of 1.5 x 0.86 capillary glass (BF150-86-10 sample glass was shipped with your puller) and ADVANCE THE PULLER BARS ALL THE WAY TOGETHER before tightening the knobs that clamp the glass. Do not over-tighten the knobs.

⚠ *CAUTION: The program HEAT value should not exceed the listed RAMP TEST value by more than 10%.*

The following shows how to load a piece of glass:

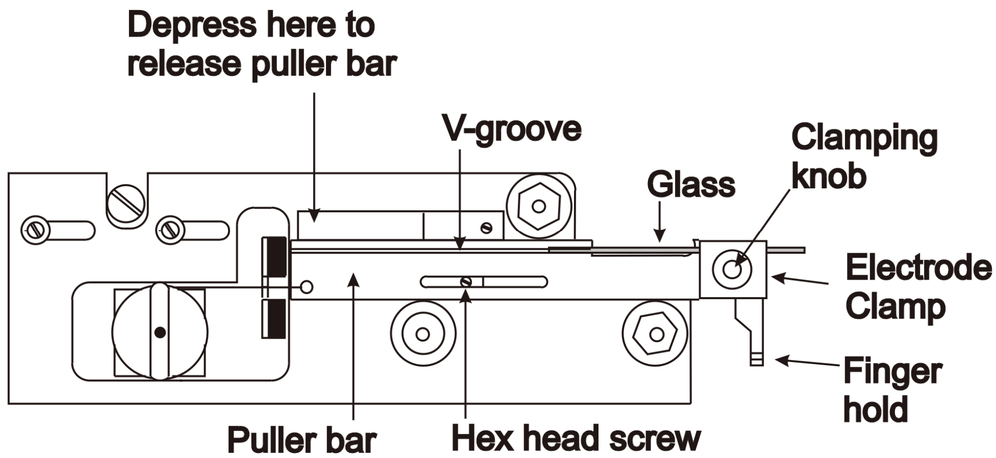


Figure 3-7. Loading a piece of glass.

a. Loosen both clamping knobs about one complete rotation to avoid breaking the glass as it passes under each electrode clamp.
b. Place glass in V-groove in puller bar, slide it beyond the clamp abouttwo centimeters and tighten the clamping knob.
c. Depress the spring stop on each puller bar to release them from their catch position.
d. Pull both bars towards each other using the finger holds. Hold the puller bars in position using the thumb and index finger of one hand. The hex head screw should be touching the end of the slot in both puller bars.
e. Loosen both clamping knobs and carefully slide the glass through the holes in the side of the heater chamber and into V-groove of opposite puller bar.
f. Tighten down both clamping knobs.

11. Press the “Pull” button on the front panel to start the RAMP TEST.

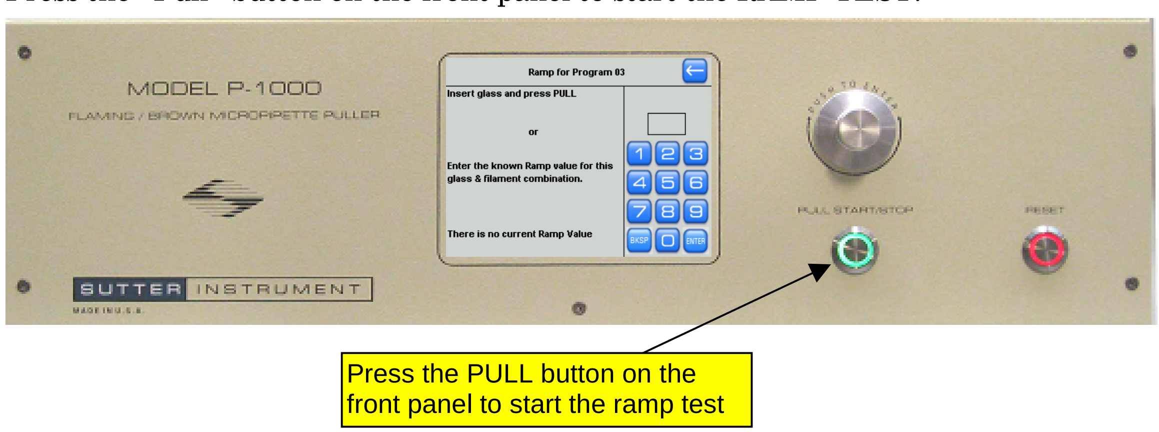


Figure 3-8. Using the PULL button to start the ramp test.

The **Ramp Test** will take a few minutes. Please wait: During the ramp test, the filament will gradually heat up until it is hot enough to melt the glass. The glass will not separate, but once the ramp value is reached, there will be a reduction (hour glass-shape) where the glass softened.

12. Once the Ramp Value has been reached, accept the ramp value.

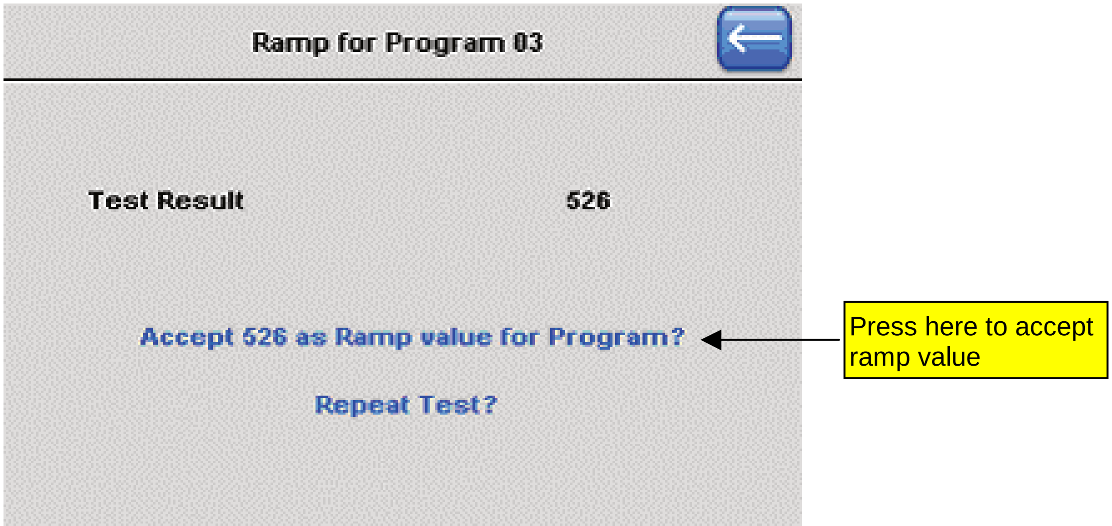


Figure 3-9. Selecting a ramp value.

13. The accepted ramp value is now installed in the top right corner of Program 3.

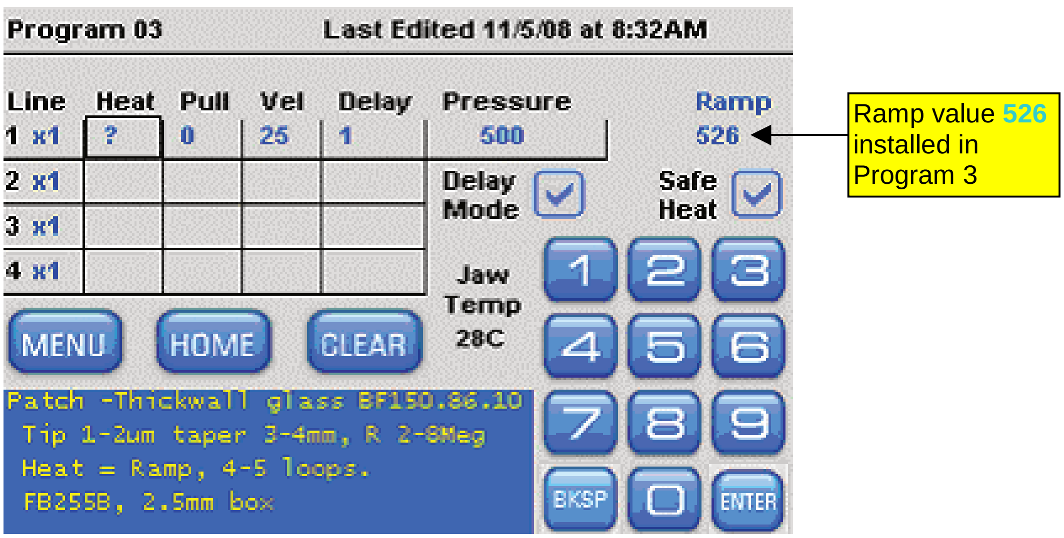


Figure 3-10. Identifying the installed ramp value.

14. Select Line 1 Heat by touching the screen. Use the keypad or knob to install the ramp value as your heat setting. If you're ramp test value is 526, install 526 for your Heat. Heat settings within 5 to 10% of the ramp value are the most stable heat settings to use.

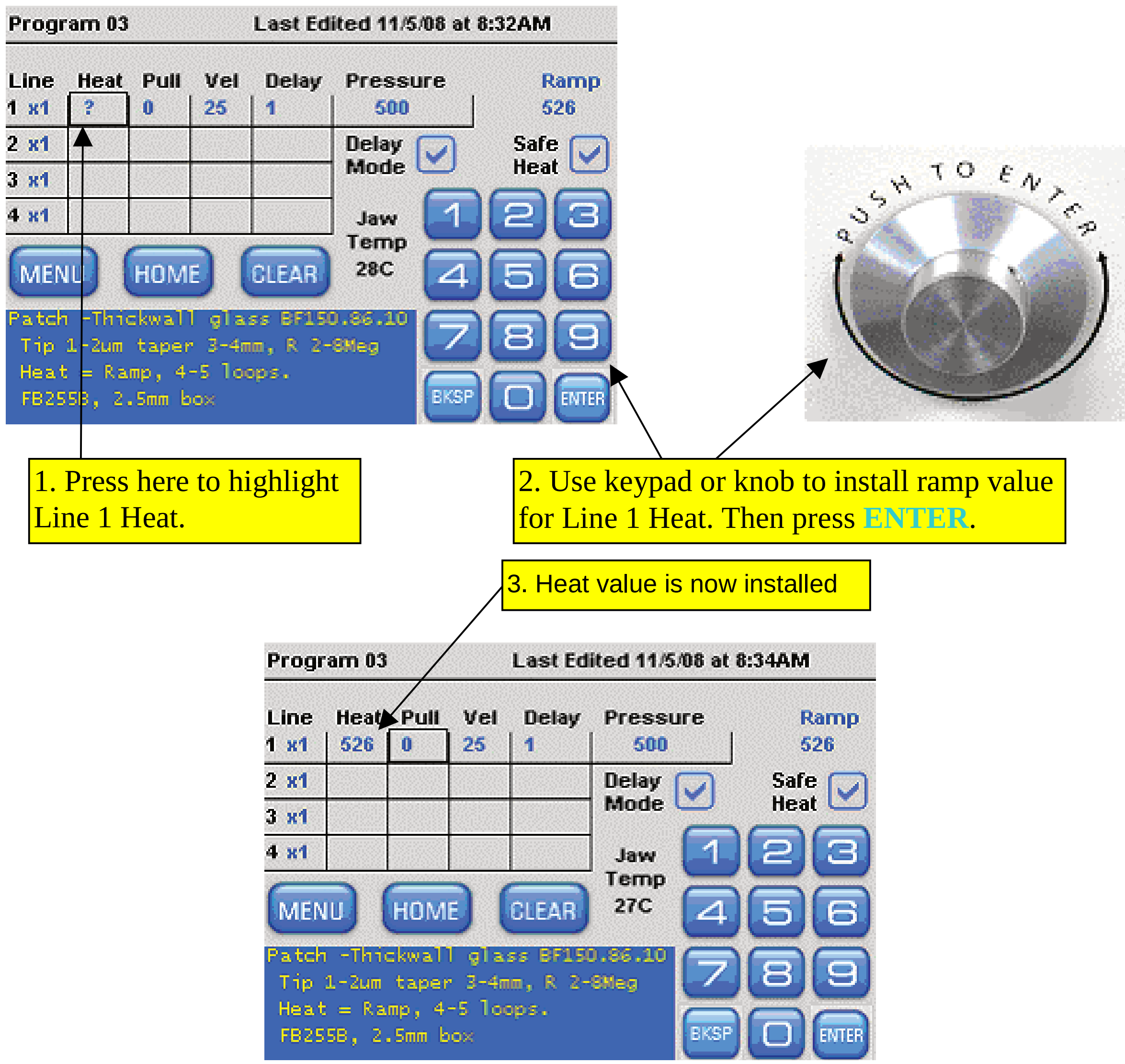


Figure 3-11. Using the installed ramp value for Heat.

15. Remove the glass that was used for the Ramp test and install a NEW piece of 1.5 x 0.86 glass into the puller bars.
16. With a new piece of glass and the Heat value installed, press the Pull button on the front panel.

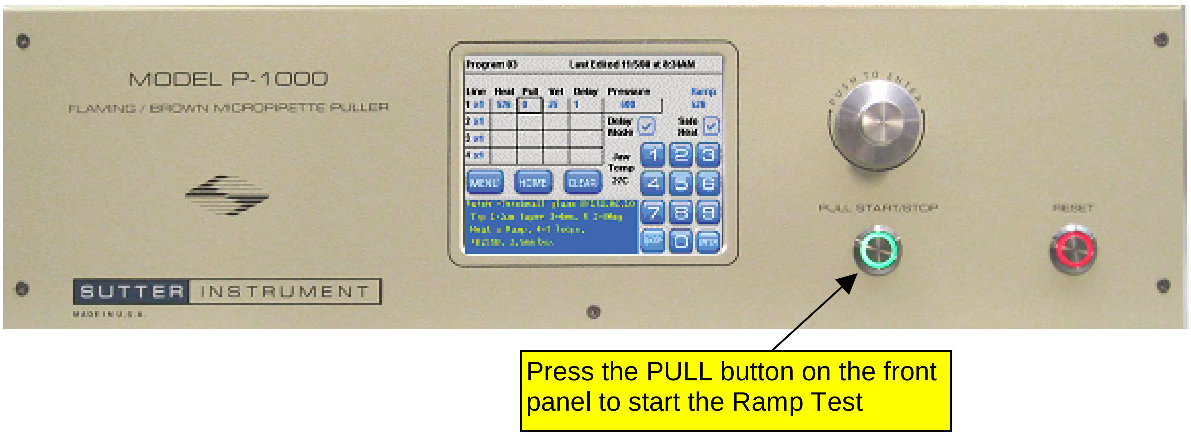


Figure 3-12. Pressing PULL on front panel to start the ramp test.

17. The puller will cycle through this one line program and pull the glass in 4-5 heating cycles (Loops).

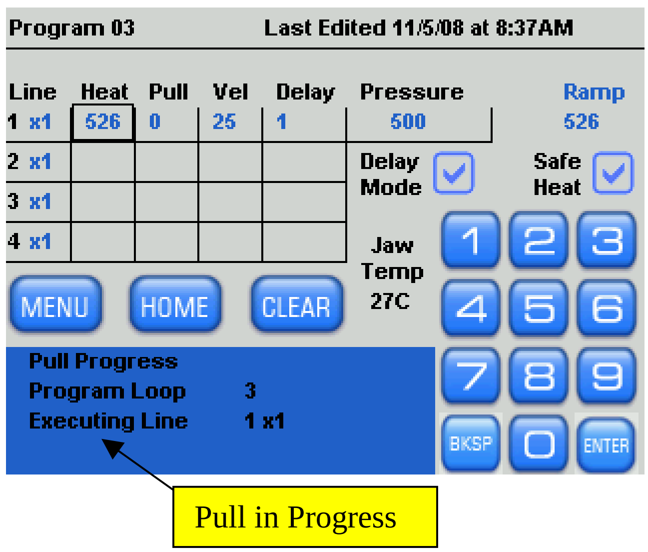


Figure 3-13. Cycling through a looped 1-line program

18. After 4-5 loops, the glass will separate making two identical pipettes. You will briefly see the pull results in the bottom text box.

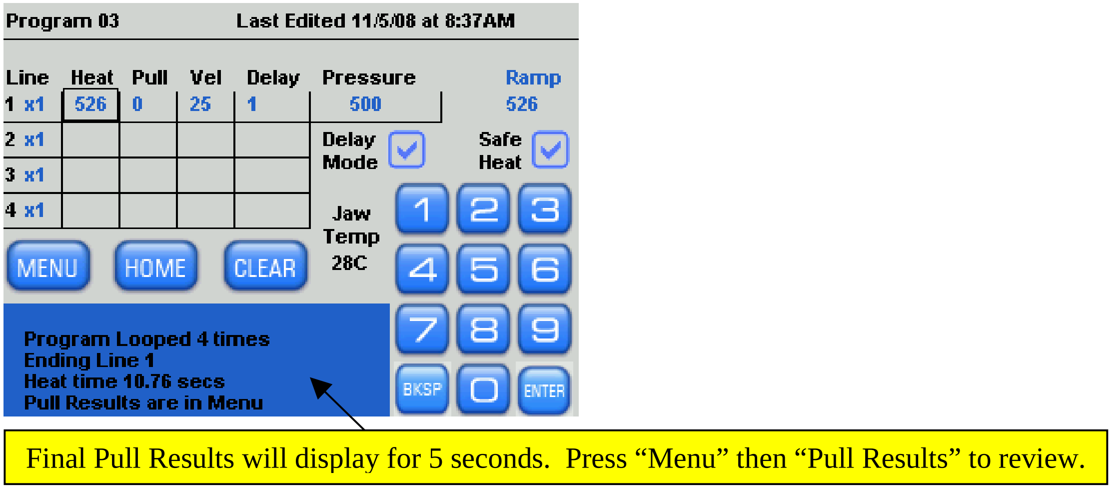


Figure 3-14. Brief display of Pull Results.

19. To make a different type of pipette, refer to the program sheet and use one of the six pre-installed programs in your puller (Program # 0-5). If your application or glass and filament combination are not found on the Program Sheet, use the Sutter Cookbook feature found in the menu on the left side of the "Home" screen to search for, and install, the appropriate program settings.

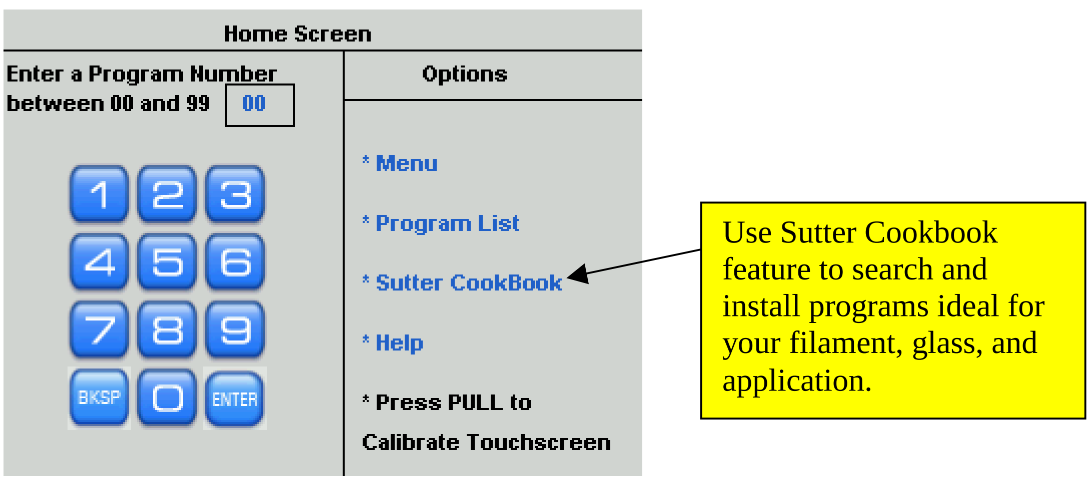


Figure 3-15. Making a different type of pipette using the Sutter Cookbook.

20. The **Safe Heat Mode** helps to prevent burning out the filament. If you install a heat setting that is 10% over or under the ramp test value, see example below, a **Safe Heat Warning** will appear in the text box and disallow that heat setting. Change the heat setting to avoid damaging or burning out the filament.

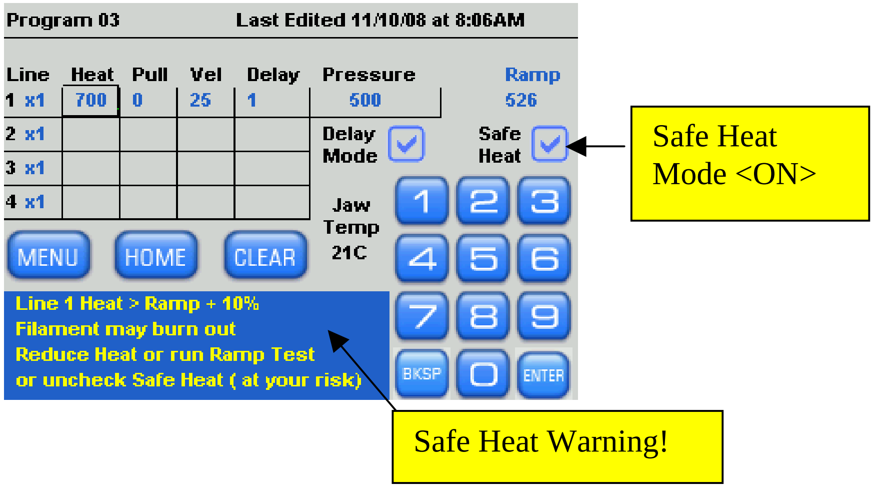


Figure 3-16. Placing puller into Safe Heat Mode.

21. In the Help Menu, you can access additional features including the Glossary, step-by-step instructions on how to install a filament, and more.

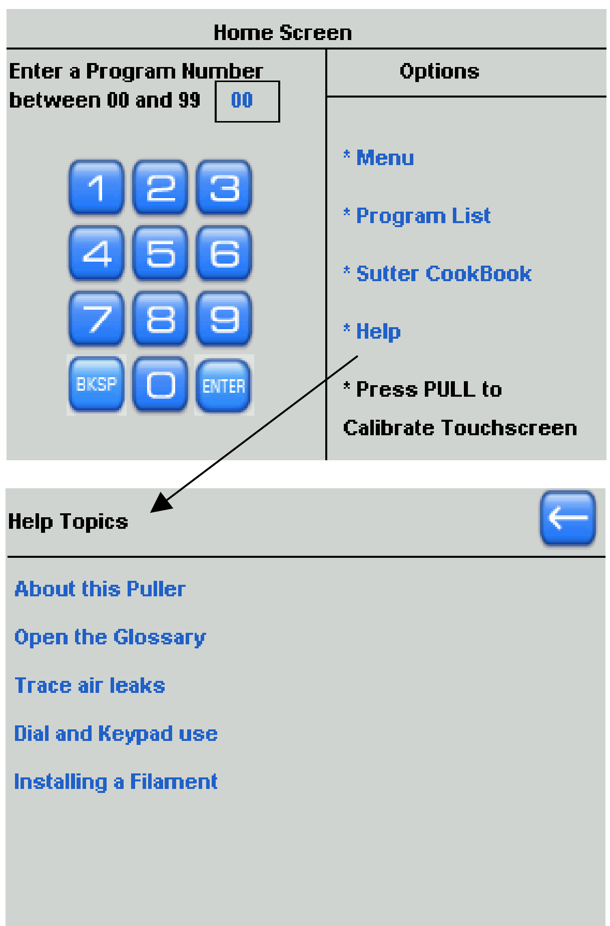


Figure 3-17. Obtaining Help information.

3.1.2 Default Configuration

Unless requesting special programming or setup at the time of purchase, the puller is setup and shipped with the following standard factory configuration:

Table 3-1. Standard factory configuration.

Heating Filament Installed	2.5mm Box (Catalog # FB255B)
Glass used to program puller (sample sent with puller)	1.0mm O.D. x 0.5mm I.D. borosilicate, with filament (Catalog # BF100-50-10)
Factory installed programs (see enclosed program sheet)	0 - Micropipette 0.06 µm tip for high Megohm recording. 1 - Patch pipette 2 - Microinjection pipette tip < 1 µm 3 - Patch pipette using 1.5 x 0.86 mm glass 4 - Pronuclear injection pipette using 1.0 x 0.78 mm glass 5 - Patch pipette using 1.5 x 1.1 mm glass

Additionally, more than 100 programs from the Sutter Pipette Cookbook are stored in the P-1000 any one of which is available can be loaded up into any program.

In describing the operation of the puller, the above configuration is assumed. If the configuration of your puller differs, the operating instructions still apply but references to specific program settings may not be accurate. Inappropriate settings will generally only affect your ability to control the geometry of the glass micropipette you are trying to fabricate. However, **the heating filament can be destroyed by an excessive value for the HEAT parameter**. We recommend you refrain from executing a program until you have read this section of the manual, have run the Ramp Test described herein, and are using the Safe Heat Mode.

Program (0-99)	A program consists of one or more lines or loops that, when executed, will 'pull' the capillary glass inserted in the instrument. A program can be up to 4 lines. Each line can be repeated up to 4 times with a maximum of 10 lines including repeats.
Write Protection (Locked/Unlocked)	A program that is Locked cannot be edited or changed. No Keypad is displayed when the program is locked. A program's locked status is changed in the Menu screen (displayed when pressing the MENU button).
Safe Heat (On/Off)	Causes a warning if a Heat value is more than 10% above or below Ramp value.
Pressure Setting (Range 0-999)	Shows the programmed value of the air pressure during the active cooling phase of the pull cycle.
Date/Time	Shows the date and time of the last program edit.

HEAT
(Range 0-999)

HEAT controls the level of electrical current supplied to the filament. The HEAT required to melt a piece of glass is a function of the filament installed and the particular glass size and composition. It is important that the HEAT value be set relative to the Ramp Test value as discussed in the Operation Section. Generally, changes to HEAT will be made in steps of about 5 units since in most cases smaller changes will have little effect.

PULL
(Range 0-255)

This parameter controls the force of the hard pull. In general, the higher the PULL, the smaller the pipette's tip diameter and the longer the taper. Useful changes in PULL strength are 10 units or more to see an effect.

VELOCITY
(Range 0-255)

The velocity of the glass carriage system is measured as the glass softens and begins to pull apart under a constant load. The increasing velocity of the initial pull is determined by the viscosity of the glass, which in turn is a function of the glass temperature. The adjustable velocity allows for a selection of a precise glass temperature as the trip point for the hard pull. Useful values for velocity range from 10 to 150 with lower values being used for patch and injection pipettes and higher values for micropipettes. **See the Programs section for a discussion of the significance of VELOCITY=0.**

TIME
(Range 0-500)

TIME is one of two available modes of cooling and controls the length of time the cooling air is active. If VEL>0 then one unit of TIME represents 1/2ms. If VEL=0 (when in fire-polish mode), then one unit of TIME represents 10ms. **See the Programs section for a discussion of the significance of TIME=0.**

Delay Mode ☐ not checked

DELAY
(Range 0-500)

DELAY is a cooling mode that controls the delay time between when the heat turns off and when the hard pull is activated. The air is automatically turned on for 300ms. The higher the DELAY value, the cooler the glass will be when the hard pull is executed. Thus, increasing the DELAY results with decreased taper length and increased tip diameter. If VEL>0 then one unit of DELAY represents 1/2ms. If VEL=0(when using fire-polish mode), then one unit represents 10ms. **See the Programs section for a discussion of the significance of DELAY=0.**

Delay Mode ☑ checked

3.2 Programs

3.2.1 Program Structure

The resulting size and shape of a micropipette made using the P-1000 is determined by the parameter values that are programmed by the user. Up to 100 separate programs can be installed and saved. Each program is structured as follows:

Program Consists of one or more Lines, each of which represents a pull cycle. When a program is run, all lines within the program are sequentially executed, beginning with Line 1. As a line is executed, the capillary glass inserted in the instrument is “pulled.” A program can consist of up to 4 lines, and each line can be repeated up to 4 times with a maximum total of 10 pull cycles for the program.

Cycle A **Cycle** consists of six programmable parameters: **Line**, **Heat**, **Pull**, **Velocity**, either **Time** or **Delay**, and **Pressure**. A **Cycle** is equivalent to one line of Program code. Each **Cycle** can be executed up to four times.

A program’s contents can be viewed by displaying the Program Edit screen for the program in question.

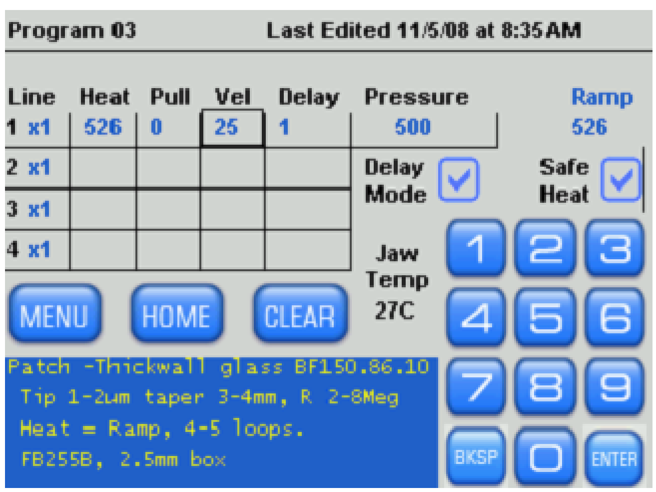


Figure 3-18. Examining the contents of a program using the Program Edit screen.

3.2.2 Program Line Pull Cycle Parameters

Each of the four programmable parameters in a program line is defined below:

HEAT

HEAT controls the level of electrical current supplied to the filament. The HEAT required to melt a piece of glass is a function of the filament installed and the particular glass size and composition. It is important that the HEAT value be set relative to the Ramp Test value as discussed in the Operation Section. Generally, changes to HEAT will be made in steps of about 5 units since in most cases smaller changes will have little effect.

PULL
(Range 0-255)

This parameter controls the force of the hard pull. In general, the higher the pull, the smaller the pipette's tip diameter and the longer its taper. Useful changes in PULL strength are 10 units or more to see an effect.

NOTE: A one-line program containing PULL = 0 and a low VELOCITY setting (15 – 40), looped 2-3 times, can be used for making short patch-type pipettes.

VELOCITY
(Range 0-255)

The VELOCITY of the glass carriage system is measured as the glass softens and begins to pull apart under a constant load. The increasing velocity of the initial pull is determined by the viscosity of the glass, which in turn is a function of the glass temperature. The adjustable velocity allows for the selection of a precise glass viscosity as the trip point for the hard pull. Useful values for velocity range from 10 to 150, with lower values (15-40) being used for patch pipettes and higher values (50-125) for microinjection pipettes. ..

VELOCITY = 0 Special Condition (Fire Polish Mode): If VEL=0 and PULL=0, the HEAT will be on for the duration of the TIME programmed (1 unit equals 10ms). This feature allows you to use the puller to fire polish the resulting patch pipette...

TIME
(Range 0-500)

Controls the length of time the cooling air is active. This parameter is one of two available modes of cooling If VEL>0 then one unit of TIME represents 1/2ms. If VEL=0 then one unit of TIME represents 10ms.

TIME = 0 Special Condition: The air solenoid is disabled when TIME=0 (no active cooling). This allows the pulling of special pipette shapes. Most often used to pull long tube-like shapes such as those used for microperfusion.

DELAY (Range 0-500)	A cooling mode that controls the delay time between when the HEAT turns off and when the hard PULL is activated. The air is automatically turned on for 300ms. The higher the DELAY value, the cooler the glass will be when the hard PULL is executed. Thus, increasing the DELAY results in decreased taper length and increased tip diameter. If VEL>0 then one unit of DELAY represents 1/2ms. If VEL=0 then one unit represents 10ms. **DELAY = 0 Special Condition:** The air solenoid is disabled when DELAY = 0 (no active cooling). This allows the pulling of special pipette shapes. Most often used to pull long tube-likes shapes such as those used for aspiration, microperfusion, or holding.

3.2.3 Pull Cycle

A typical pull cycle in a program line is described below:

1. The heat turns on.
2. The filament heats up, the glass softens, and a weak pull draws the glass out until it reaches the programmed velocity.
3. When the programmed velocity has been reached, the heat turns off, and the air turns on to cool the filament and glass.
4. If TIME is greater than 0 (zero), the hard pull (if any) is executed after a short delay and then the air is activated for the specified TIME.

   If DELAY is greater than 0 (zero), the air is activated for a short period and then the hard pull is activated after the specified DELAY.

3.2.4 Start Up.

After switching on or after pressing RESET, the P-1000 tests the air and heating systems.

The opening screen appears next

Note on Entering Numbers: Numbers can be entered with the on-screen touch Keypad or with the Rotary Dial. Key in all digits for a field (e.g., “012” for a three digit field or key in “12” followed by pressing ENTER. You can also turn the Rotary Dial to increase or decrease a value then press Enter.

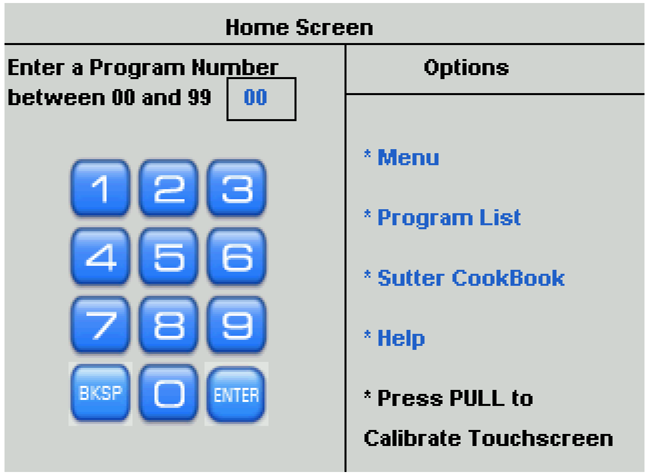


Figure 3-19. Home screen.

To open one of the 100 programs, use the touchscreen keypad or the rotary dial to select and enter a program number.

The display will appear as shown below in the following figure.

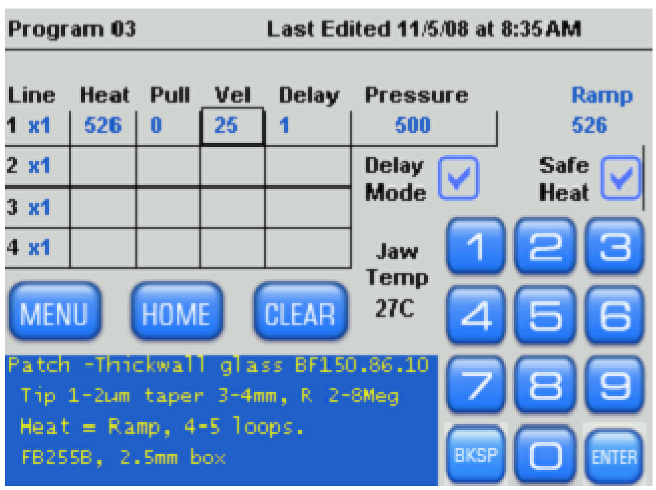


Figure 3-20. Examining the contents of a program using the Program Edit screen.

On this screen you can:

- Press  to open the Menu Screen.
- Press  to return to the opening Home screen, where another program can be opened.
- Press  to erase all parameter values (with the exception of all text in the Notes field), and sets the pressure to a default value of 500. You are asked to confirm the erasure.
- **Safe Heat:** When checked (), a warning is given if Heat values differ from Ramp by more than 10%. If there is no Ramp value and the Safe Heat is on, the warning is given upon opening the program.

- **Notes:** The blue text box at the bottom of the screen can be used to record any information (e.g., glass and filament for the program, your name, expected results, etc.). To edit, just touch the blue text box and an edit screen with a keyboard will appear.
- **Pull pipettes.** Once you are satisfied with the settings within the displayed program, you use this program to pull pipettes. To begin the pull process, press the Pull Start/Stop button on the front panel.

An alternate way in which a program can opened for viewing or editing is by pressing the Program List in main menu, and then selecting the program in the list shown in the following screen.

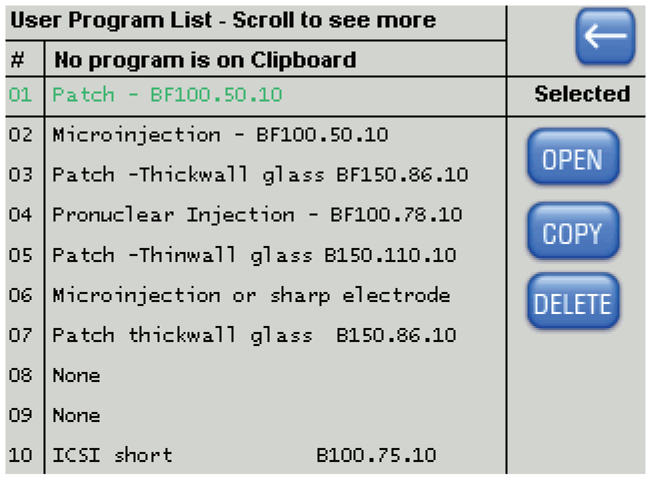


Figure 3-21. Program list screen.

Use the Knob on the front panel to scroll through the list. The currently selected program is shown in green. Press either the OPEN button or the Knob on the front panel to open the program, whereupon the Program Edit screen will be displayed showing the details of the selected program.

3.2.5 Editing a Program

On opening a program, the Line 1 Heat value is highlighted with a blue rectangle. You can touch and highlight any parameter for editing. A highlighted parameter can be edited using the Keypad or the Dial. After a number is selected and the ENTER button is pressed, the next field will be highlighted.

3.2.5.1 Editing Tips

- All changes to a program cause the “Last Edited” time to update (top of the screen).
- A warning is given if a Keypad entry exceeds the maximum for that parameter.
- If Safe Heat is checked (☑), a warning is given if a Heat value differs from Ramp by more than 10%.

3.2.5.2 Edit User Notes

By touching the blue text box at the bottom of your program you can bring up the “Edit User Notes” Screen.

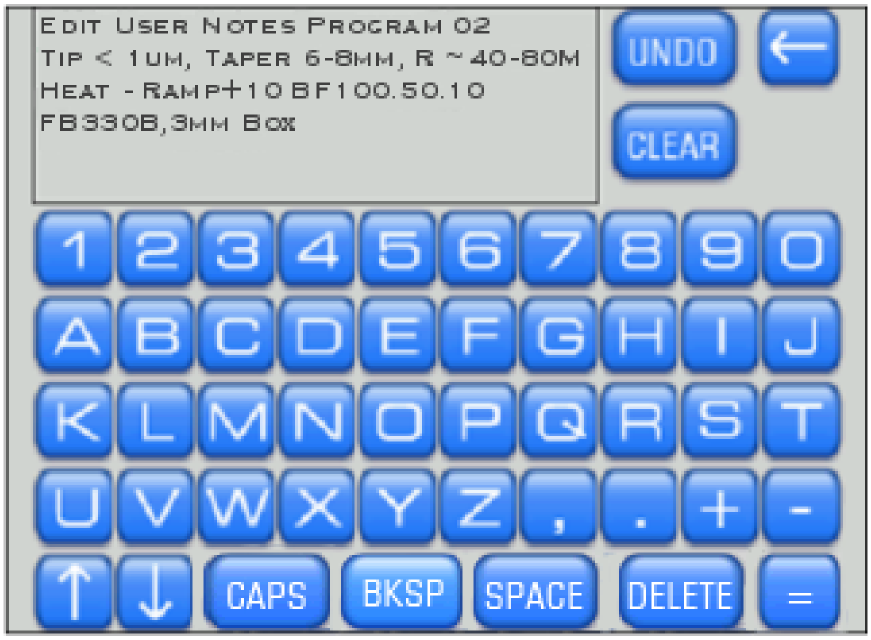


Figure 3-22. Edit user notes.

Here you can edit the notes, press [Undo] to start over, or [Clear] all text and write your own notes. Pre-installed factory programs (0-5) and any programs imported from the Cookbook feature will have pre-written text notes detailing the filament, glass, pipette type and appropriate heat to use with that program. Take caution before clearing all text, as you might want to retain some information already describing this program. If the notes no longer apply to your program, clear all text and write your own notes.

The "Notes" section is a good place to record whose program it belongs to, the filament type (box or trough), the glass size (OD/ID), and the application for which the program was designed. You might also want to record the heat setting you are using in relation to the Ramp Value (e.g., Heat = Ramp +10).

The Rotary Dial allows cursor movement through the text. Cursor position is indicated by the character in which the cursor is positioned turning green.

3.3 Menu screen

3.3.1 General

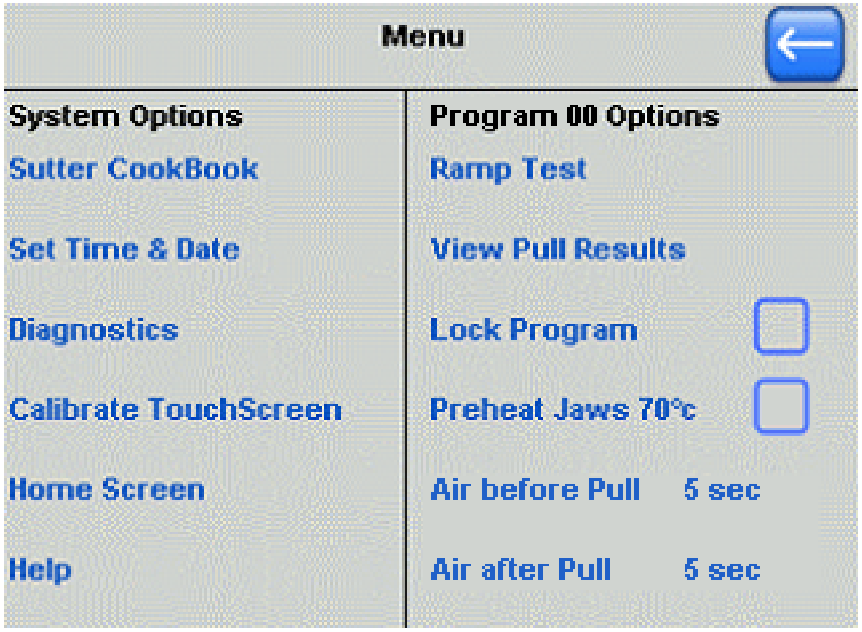


Figure 3-23. Menu screen.[1]

3.4 System Options

- Sutter Cookbook: Refer to the Cookbook section for information on how to import an appropriate program for your application.
- Set Time and Date: This is the system clock used to record the time of when the program was installed or last edited.
- Diagnostics: Each of the P-1000 hardware components can be individually operated to find any malfunction.
- Calibrate Touchscreen: Recalibration may be needed due to drift over time. If calibration is too far off for the touchscreen to work, press RESET and calibrate at the Home screen
- Home Screen: Opens the Home screen.
- Help: Opens the Glossary and Help files

3.5 Program Options

- Ramp Test: Opens the Ramp Test screen
- View Pull Results: Opens a screen that shows details of the last two pulls.

[1] NOTE: The program option "Preheat Jaws 70˚C"in the Menu screen is associated with the Thermolock™ feature, and exists only in P-1000 systems that shipped on and after November 29 of 2010.Also, in the same systems, the Menu screen has both columnar titles ending in "Options", replacing the previous "Operations".

- **Lock Program:** If checked ( ), the current program is locked.
- **Air Before Pull:** This function defines the time taken to flush the air subsystem with dry air before the pull begins. The default setting is 5 seconds.
- **Air After Pull:** Same as above, but after the pull is finished. The default setting is also 5 seconds.

3.6 Ramp Test

The HEAT value required to melt your glass is based on the characteristics of the heating filament that is installed and the OD/ID of glass you are using. The RAMP TEST allows you to systematically establish or adjust program HEAT values as a function of the filament/glass combination. This test should be run when using the puller for the first time, before writing or editing a program, whenever you change glass or whenever you change the heating filament. A HEAT setting equal to the RAMP value is the best first setting with which to start.

The ramp value is associated and stored with the current program.

You can access the Ramp Test screen from the Menu screen or by touching the Ramp parameter in the Program Edit screen, whereupon the following screen is displayed.

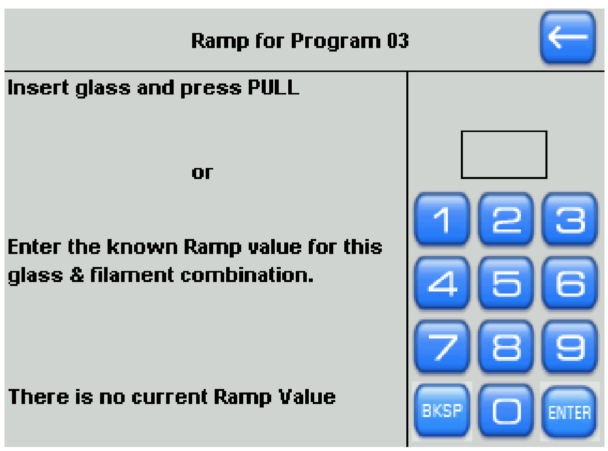


Figure 3-24. Ramp test ramp value screen.

If you are using a filament / glass combination for which you have already run a Ramp Test, you can simply type or dial it in. Otherwise, follow the instructions on screen.

1. When the above screen is displayed, press PULL to run the ramp test.
2. The puller increments the HEAT, and the filament, after 1-2 minutes, begins to get hot and glows orange in color.
3. The puller bars begin to move apart once the HEAT is hot enough to soften the glass.
4. As the glass begins to soften, a certain velocity of the puller bars moving apart is detected, at which point the heat turns off.
5. Once there is a small melt in the glass, the new ramp value will be displayed.
6. You can accept the ramp value (in which case the value gets installed into your program), repeat the ramp test, or ignore it and go back.

7. Accept the ramp value and use a heat-setting equal to, or within five (5) units of, the ramp test value. This heat setting will be a stable and safe heat to use—one that will not create instability or damage the filament.

3.6.1 Heat Value Recommendations

For Trough filaments:	Recommended starting value: Ramp + 5 units
	Maximum program HEAT value(s) = Ramp value + 10%
For Box filaments:	Recommended starting value: Ramp value = HEAT setting.
	Maximum Program HEAT value(s) = Ramp value + 10%

3.7 Pulling Pipettes

3.7.1 Procedures

Prior to pulling a pipette for the first time, it is important to establish what HEAT value is appropriate to melt your glass. Before executing a program for the first time, **run the RAMP TEST** as previously described, and accept the Ramp value into the program. The HEAT settings in the factory-installed programs are typically set at Ramp value. If your Ramp test value differs from the factory Ramp value, adjust the HEAT in your programs to your Ramp value.

Once you have adjusted the HEAT value relative to the Ramp value, pulling a pipette is very straightforward. Try executing the factory installed programs with the sample glass to become acquainted with the pulling process.

1. Load the glass into the puller as described previously in the FIRST TIME USE chapter.
2. Press  and then 

, to open Program 0.
3. Inspect the parameter values displayed for Program 0. Program 0 should display the factory-installed values listed on the enclosed program sheet. Adjust the HEAT setting to your Ramp value.
4. Press the < PULL > button. The heating filament will turn on and the glass should separate within 10 seconds. The display will then report the number of heating cycles and the total time that the heat was turned on.
5. Loosen the clamping knobs and remove the pipettes from the puller bars.

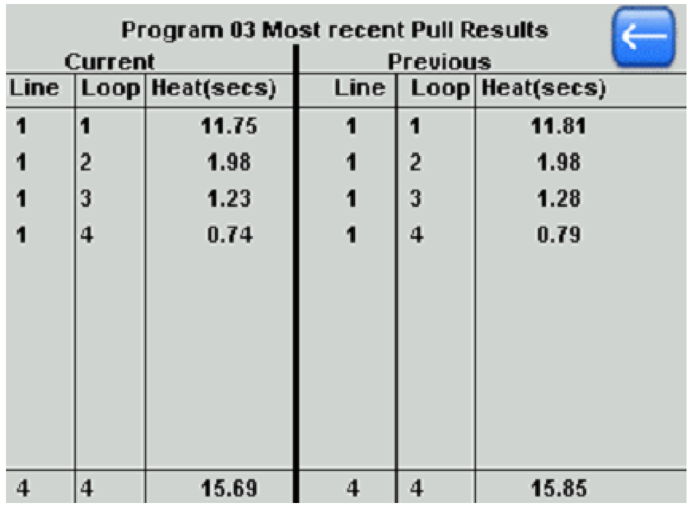


Figure 3-25. Pull cycle report screen.

Unless otherwise stated on your program sheet, Program 0 is factory pre-programmed to pull a micropipette (tip diameter less than 0.1mm) from 1.0 x 0.5 mm borosilicate glass. It will pull the pipette in one heating cycle. The time reported is very useful for developing programs and will be discussed in the Parameter Adjustment section of this manual.

A feature of the P-1000 is its capability to loop through a program. This is demonstrated using Program 1, which is a factory pre-installed one-line program "loop" (repeat and pull in stages) to create a patch-type pipette with a short taper and 1-3μm tip. Press RESET to exit Program 0, and then 

, 

 to open Program 1. The display for Program 1 should read similar to the following:

Figure 3-26. Sample program.

Load glass into the puller and press <PULL>. The heating filament should loop on and off repeatedly. Once the glass has separated, the pull is complete, and the display should read similar to the following:

Program 03 Most recent Pull Results

Current			Previous		
Line	Loop	Heat(secs)	Line	Loop	Heat(secs)
1	1	11.75	1	1	11.81
1	2	1.98	1	2	1.98
1	3	1.23	1	3	1.28
1	4	0.74	1	4	0.79
4	4	15.69	4	4	15.85

Figure 3-27. Pull cycle report (multiple-loops).

After the heat turns on in line 01, the glass heats up and draws apart until it reaches a VELOCITY of 30, at which point the heat turns off and the cooling air turns on. The puller is “aware” of the fact that the glass has not separated yet, and will go back to line 1 of the program and try again; in effect, it begins ‘looping’. It will continue to do so until the glass separates. This looping capability is particularly useful for fabricating patch pipettes, which require multiple heating cycles to form the characteristic stubby geometry.

Note: **The results for the last two pipettes pulled are available from the Menu screen. The Heat-on Time for each line of program executed is recorded.**

3.7.2 Notes on Program Operation

There is always the possibility that the puller will be given a set of values that ‘stall’ its operation. For example, the HEAT value has not been set high enough to melt the glass, thus the glass cannot be pulled and no VELOCITY can be achieved. If it appears that a situation of this type has arisen, press the STOP key. This action aborts program execution and allows editing to take place. The safe-heat mode (turned on) should prevent this event from occurring.

All programs entered into memory (maximum of 100) remain there even after the power is turned off or the RESET switch is pressed. Nevertheless, it is strongly suggested that you keep a written record of your programs in case of unexpected difficulties.

3.8 Parameter Adjustment

3.8.1 General Information

Micropipette and microinjection needle programs are sufficiently different from patch pipette programs that the following information on parameter adjustments has been divided into three sections: **Micropipette/microinjection needle fabrication**, **Patch pipette fabrication** and **Technical Tips**. Even if your research only requires one type of pipette, we recommend that you read all three sections for full appreciation of the capabilities of the puller. Please note that the programs referred to in the following text are not necessarily meant to pull functional pipettes, but are intended as an exercise to help develop an understanding of the programming process. Unless otherwise stated, parameter adjustments assume that the puller is in the **TIME** mode of active cooling.

3.8.2 Micropipette/Microinjection Needle Fabrication

Consider the following programs using a 2.5 x 2.5 mm box filament:

Sharp Microelectrode Program using 1.0 x 0.5 mm borosilicate glass

HEAT	PULL	VELOCITY	TIME	PRESSURE
Ramp	150	75	250	500

Microinjection Pipette Program using 1.0 x 0.78 mm borosilicate glass

HEAT	PULL	VELOCITY	DELAY	PRESSURE
Ramp	60	80	90	200

3.8.2.1 HEAT

The HEAT setting will affect the length and tip size of the pipette. In general, higher HEAT settings tend to give longer and finer tips. For trough filaments, the recommended starting HEAT value is the ramp test value plus 10%. For box filaments, the recommended starting HEAT value is the ramp test value. The program listed above will typically have heat on for 5 to 8 seconds after the **<PULL>** button is pressed. If the time is longer than eight seconds, and you are trying to pull a fine micropipette, increase the HEAT in 5 unit increments until the pull takes place in less than eight seconds. If the pull occurs in less than three seconds, decrease the HEAT until the pull takes place in 4 to 8 seconds. For the best micropipette reproducibility with the finest tips, you should select a HEAT value that melts the glass in 5 to 6 seconds. For microinjection pipettes, select a HEAT value that melts the glass in about 7 seconds or longer.

3.8.2.2 PULL Strength

Low values of PULL strength in the range of 50 to 75 will give larger tips appropriate for injection needles, while 80 to 150 give smaller tips appropriate for sharp microelectrodes. The PULL strength can be set to any value desired with no danger of damaging the instrument.

3.8.2.3 VELOCITY (Trip Point)

The VELOCITY value determines the point at which the heat is turned off. VELOCITY reflects the speed at which the two puller bars are moving during the weak pull. The lower the VELOCITY value, the slower the speed of the bars when the trip point occurs. Although VELOCITY can safely be set to any value from 1-255, all values over a maximal trip point (usually about 150) will produce equivalent results. As the pull progresses, the speed of the puller bars, as measured by the velocity transducer, reaches a point where further increases in the VELOCITY trip point will not change the time point at which the heat is turned off. VELOCITY is typically set between 80 to 90 for microelectrodes or 50 to 80 for microinjection pipettes.

In a multiple cycle program, it is possible for the glass to separate before the trip velocity is attained. Thus, the glass is subjected to heating as it separates. Such an occurrence can lead to difficulties in forming tips as well as lack of reproducibility. If you are using a one-line, looping program, try decreasing the VELOCITY a few units at a time. If your program is a multi-line program, decrease the VELOCITY in the next to last line of the program. Decreasing the VELOCITY will increase the amount of glass left in the last cycle of the program, thus allowing the glass to attain the trip velocity before separating.

3.8.2.4 <u>TIME Mode (Cooling)</u>

The TIME parameter controls the length of time the cooling air is active (one unit of TIME is equivalent to 0.5 ms). In order to produce effective cooling, the air must be supplied to the filament and glass during the time the tip is being formed. When pulling sharp electrodes, the hard pull lasts several tens of milliseconds. Because of this fact, increasing cooling TIME values beyond a certain range (typically 200 to 250) will have no effect. Values of TIME under 200 will cool the glass less as the tip is being formed and lead to a longer taper. However, once TIME values become too short (values in the range 110 to 130) cooling becomes ineffective. The glass will not form a tip and instead forms a wispy fiber. The very finest tips for a given PULL and HEAT will be formed at an air setting of about 5 units higher than the lowest TIME value that forms a tip. Because of this quite narrow working range of usable TIME values for making micropipettes, it is not recommended to vary cooling, and therefore electrode tip length, by adjusting TIME. Adjusting the cooling air pressure and/or switching to the **Delay** mode of active cooling are both more effective means of controlling tip length (see below).

3.8.2.5 <u>Delay Mode (Cooling)</u>

In **TIME** mode, especially when using larger and or thicker walled glass, active cooling may not be sufficient to produce short pipette tapers. This may even be true at increased PRESSURE settings (i.e. >500). In this case, it is recommended that the Delay mode of active cooling be used. The method for changing to the Delay mode is described under “Software Control Functions” and a brief description of the two modes of cooling is given in “Programs”.

After switching to DELAY mode, one then has direct control over the delay between turning the heating filament off and initiating of the hard pull. Because the cooling air is turned on when the filament is turned off, increasing DELAY profoundly increases glass cooling before and during the hard pull.

With a range of control over the degree of pipette cooling one can control the rapidity of pipette taper. Higher DELAY values (longer delay) increase cooling and form a pipette with a more rapid taper (shorter shank) while lower DELAY values (shorter delay) decrease cooling and form a pipette with a more gradual taper (longer shank). Fortunately, cooling induced changes in pipette taper generally occur with little effect on tip size. This is quite valuable as the resistance of pipettes is a strong function of the length of the taper. By making a pipette with a sharper taper, one can often decrease pipette resistance significantly without changing the size of the pipette tip. Furthermore, the ability to control tip length is invaluable for experiments where long tips are necessary for penetration into deep tissues or where short stiff tips are necessary for adequate beveling.

Minimum and maximum useable values of DELAY can be expected; their exact values are dependent on glass thickness and diameter. If DELAY is too short, the glass will not cool sufficiently to form a tip and a long wispy fiber of glass will be formed. Values under 40 units tend to be ineffective. At some maximal value of DELAY, the glass may be cooled too much to separate during the hard pull. Under these conditions, the puller will typically execute multiple cycles in order to separate the glass and the glass may break at a large tip diameter. Maximum usable values of DELAY will be dependent on glass dimensions but are expected to be near 200 (100ms of delay).

3.8.2.6 PRESSURE Adjustment

The pressure setting controls the pressure of the cooling air delivered to the filament. The higher the pressure, the shorter the pipette taper will be. Because thin walled glass cools more rapidly than thick walled glass, the recommended values are ≤ 300 for thin walled tubing and 500 for thick walled or standard walled tubing. By varying PRESSURE around these values, the user can control pipette tip length over a moderate range.

3.8.2.7 Filament Width

Further control over pipette tip length can be accomplished by varying filament width. Longer tips can be formed by using wider filaments and conversely shorter tips can be formed by using narrower filaments.

3.8.3 Patch Pipette Fabrication

Micropipettes used for the electrophysiological recording technique of “patch clamping” are generally characterized by short, stubby shanks and relatively large diameter tips (> 0.7 mm). Programs that can fabricate a pipette with these characteristics generally differ from programs for micropipettes in three ways:

The trip velocity is lower.

Hard pull is not activated (PULL = 0).

More than one heating cycle is used.

The P-1000 can be used very effectively for this type of processing. The following general information will familiarize you with the effect of adjusting each of the pull cycle parameters in a typical patch pipette program. Following this general information are step-by-step instructions intended to help you establish a stable program to pull patch type pipettes and should be followed in the order described.

Consider the following sample patch program for 1.5 mm O.D. by 0.86 mm I.D. borosilicate glass using a 2.5 x 2.5 mm Box filament:

HEAT	PULL	VELOCITY	DELAY	PRESSURE
Ramp	0	23	1	500

A program sequentially executes each line of code, then loops back to the start, and begins again until the glass separates. A single line program such as this may execute 2 to 4 times before the glass separates.

3.8.3.1 HEAT

The actual HEAT value used should be sufficiently high to allow the glass to melt in the first cycle in 5 to 15 seconds. Using a higher HEAT that melts the glass in less than 5 seconds will cause no problem in the first heating cycle, but may heat the glass so much in subsequent heating cycles, that the air cooling will be less effective.

3.8.3.2 PULL Strength

A constant gravitational pull on the puller bars can be felt when loading the glass. This pull is usually adequate to form relatively small-tipped pipettes (0.5 mm). Eliminating the hard pull from the program (PULL=0) is recommended for most patch pipettes. If smaller tips are required, a moderate PULL (25-50) may be used in the last line of a multi-line program (see below).

3.8.3.3 VELOCITY (Trip Point)

VELOCITY determines the point at which the heat is turned off. If the value is too high, the glass will separate after the third heat cycle. As the VELOCITY is decreased, the amount of glass drawn-out in a given cycle will also decrease, and more cycles will be required to form a tip. The greater the number of cycles, the larger the tip and the shorter the taper will be. However, too many cycles can lead to variability. In general, it is advisable to keep the number of heating cycles to 5 or less.

3.8.3.4 Cooling

TIME Mode: When using thin-walled glass to make patch pipettes (slice patch), it is advisable to keep the TIME between 200 and 250 to maximize the cooling of the glass.

DELAY Mode: The DELAY mode (DELAY = 1) is recommended when pulling a patch pipette and using thick-walled glass, a box filament, and/or needing short-tapered pipettes. The DELAY mode provides a set 300 milliseconds of cooling.

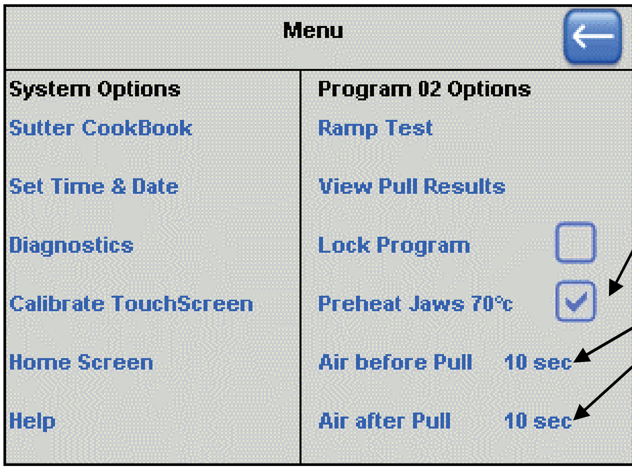
3.8.3.5 PRESSURE

The recommended pressure setting when using thick walled glass is 500 or greater. For thin walled glass, the recommended range is 200 to 500.

3.8.4 Thermolock™ Pre-Heat Mode

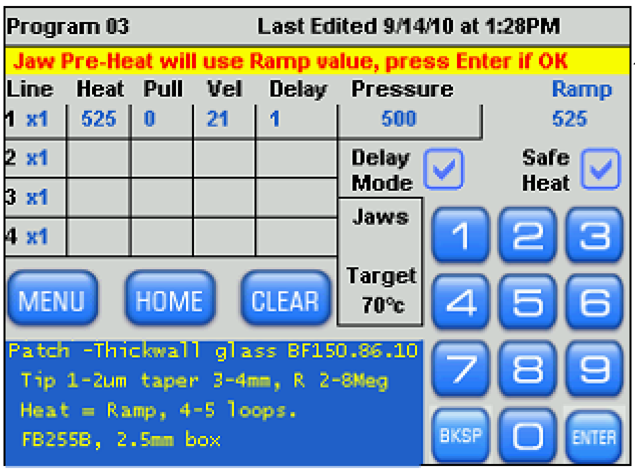
When pulling pipettes where the pull occurs in multiple stages (i.e., patch pipette programs), the temperature of the jaws that hold the filament can range from 23°C to 85°C. This variation in jaw temperature can sometimes create instability in the program and lead to inconsistency in the tip size, taper length, and cone angle of the resulting pipette. To further regulate the pulling conditions, the new Thermolock™ feature can be used to pre-heat and maintain the jaw temperature at 70°C.

If the "mid-point velocity" for writing a stable patch pipette program (Pipette Cookbook, Pg. 30) has been previously established, the shift and rise in jaw temperature is less likely to introduce variability. However, if a program remains unstable where the first few pipettes pulled are not ideal and later pulls provide better electrodes, the Pre-Heat mode for regulating the jaw temperature can make a program more stable and improve the outcome of resulting pipettes.



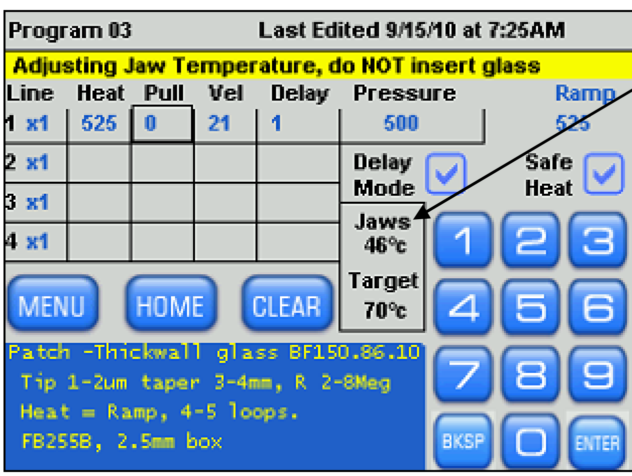
To activate the Pre-Heat mode in your program, press <MENU> and then select the box to "Pre-Heat Jaws 70°C".

When using the Pre-Heat mode, air flush durations before and after the pull are increased to 10 sec.



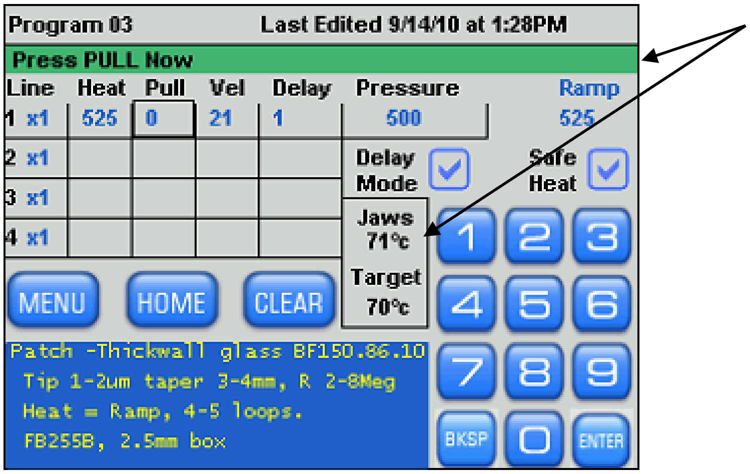
A caution is displayed indicating that the filament will light up and pre-heat. This yellow message bar is displayed when powering up and entering a Pre-Heat program; when waking from sleep; and when going from a non-Pre-Heat program into a Pre-Heat program.

During Sleep mode, the pre-heating is suspended. Upon waking from Sleep, the yellow message bar is displayed.

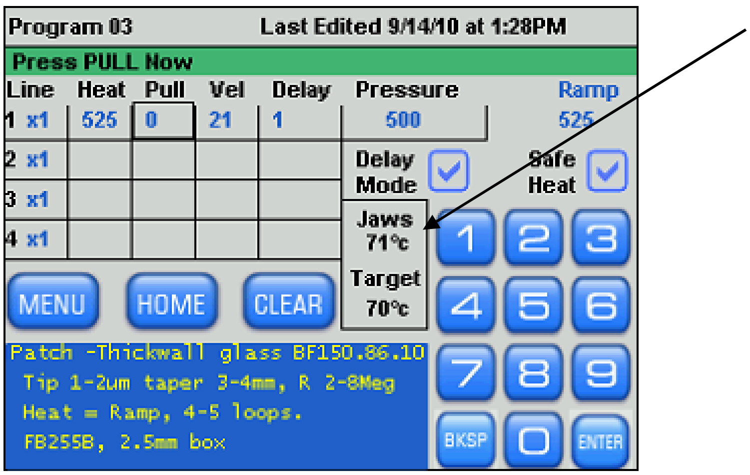


The initial pre-heating of the jaws will take a few minutes, during which the jaw temperature is continuously displayed.

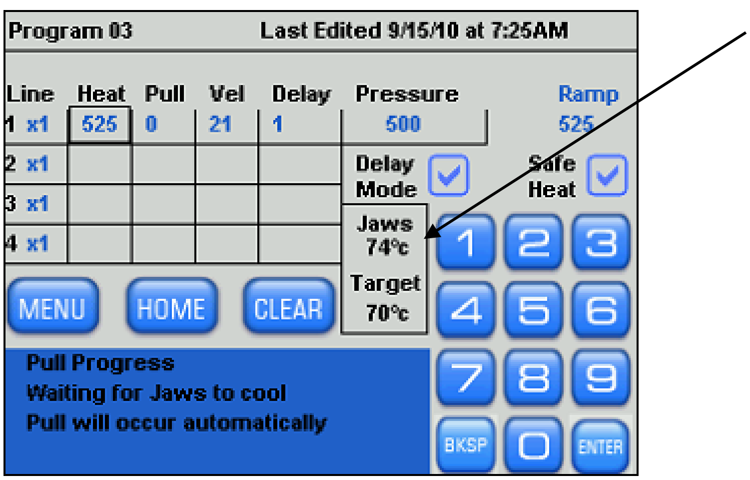
The user can load the glass at any time, but if the glass is loaded before the jaws reach 70°C, the Pre-Heat mode might not function as intended.



When the jaw temperature reaches 70°C, a green message bar is displayed indicating that it is okay to install the glass and pull a pipette.

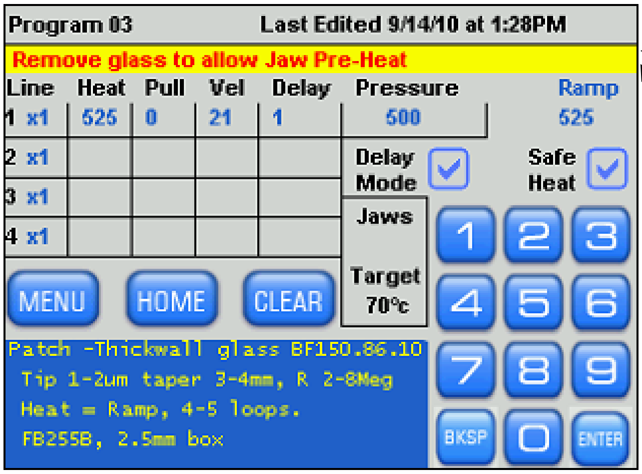


When the capillary glass is inserted, even if the Pre-Heat temperature has not been reached, the filament heat is turned off to prevent the glass from melting and/or sticking to the filament.



If the jaw temperature is above 70°C when <PULL> is pressed, the air flush occurs, but the pull cycle is delayed until the jaws cool down to 70°C.

If the jaw temperature has dropped to 70°C after the air flush, the pull cycle will begin immediately.



If a program is opened with the glass already installed, the user is requested to remove the glass to allow the Pre-Heat to continue.

If the glass is inserted when the display is in the Sleep mode, this message is also displayed upon waking from Sleep.

3.8.5 Step-by-Step Patch Pipette Programming

Run a Ramp Test with the glass you intend to use for your particular application. Refer to the manual if you need to review the Ramp Test procedure. When you know the Ramp value (R), use it in the following program.

1. Program one line of code as follows:

Filament Type	HEAT	PULL	VELOCITY	TIME/DELAY
Box	Ramp	0	*30	DELAY=1
Trough	Ramp + 15	0	*50	TIME=150

   * The VELOCITY value will need to be manipulated.

   PRESSURE should be set to 500 for thick walled glass and 300 for thin walled glass.

2. Insert your glass and execute the above program. The program should “loop” a multiple number of times (i.e. the same line will be repeatedly executed). The display will report the number of loops at the end of the pull sequence. This “looping” is the key to forming patch pipettes. For thin walled glass, 2 to 3 loops are typically all that is required. For thick walled glass, 4 to 5 loops are typically required.
3. Increase the VELOCITY in one unit increments for thick-walled glass and three unit increments for thin-walled glass. Pull a pipette after each adjustment. Note the change in the number of loops and note the geometry of the pipette (viewed with microscope). As the VELOCITY increases, the number of loops decreases.
4. Repeat step (3) only this time decrease the VELOCITY. As the VELOCITY decreases, the number of loops increases.
5. By adjusting the VELOCITY as described, establish the number of loops required to approximately form a pipette with the characteristics you desire. Set the VELOCITY value in your program to the number that falls midway between the values required to loop one more and one less times than the desired number. This is called the “mid-point velocity”. For example, while experimenting with VELOCITY values, you find that when the glass separates after 3 loops the resulting pipette looks pretty reasonable. Let Y be equal to the VELOCITY value that results with the glass separating after 4 loops. Let Z

be equal to the VELOCITY value that results with the glass separating after 2 loops. Set your program VELOCITY, to a value midway between Y and Z. This value will be a very stable VELOCITY value and will provide you with the most reproducible results.

6. The one line program just established may be sufficient for your application. However, changes made in a one-line program are amplified throughout the cycle, potentially producing gross changes in the pipette. If you need to make fine adjustments to the pipette geometry, then you should use a multi-line program. The multi-line program is based on the one line program just established. It is developed as follows:
7. Write your one-line, looping program out into an equivalent multi-line program with the number of lines equal to the number of loops.
   For example, a one line, 4-loop program with the following values can be written:

Line	Loops	HEAT	PULL	VELOCITY	DELAY
1	4	525	0	23	1

The above 1-line, 4-loop program could be written into an equivalent 4-line program, as follows:

Line	Loops	HEAT	PULL	VELOCITY	DELAY
1	1	525	0	23	1
2	1	525	0	23	1
3	1	525	0	23	1
4	1	525	0	23	1

Now, you can make adjustments to the last or next to last line to fine-tune the program and the resulting pipette.

Recommended changes to fine tune the multi-line program:

For larger diameter tips	Decrease HEAT in **last line**.
For smaller diameter tips	Increase or decrease VELOCITY in **next to last** line by 2 to 3 units, or increase/decrease VELOCITY in **last line** by 2 to 3 units, or add a small amount of PULL (10 to 20) to **last line**.

3.9 Technical Tips for Pulling Micropipettes

3.9.1 Regulating the Time it takes to pull a Sharp Pipette

3.9.1.1 HEAT

For 1.0mm O.D. tubing, if the pull takes longer than eight seconds and you are trying to pull a fine micropipette, increase the HEAT. To do this in a methodical fashion, increase the HEAT value in five unit increments, each time monitoring pull time until the pull takes place in less than eight seconds.

If the pull occurs in less than three seconds after you start, decrease the HEAT value in a similar fashion.

For 2mm O.D. tubing, the pull should occur between 10 and 15 seconds after the start. Make corrections as outlined above for smaller tubing.

3.9.1.2 Pipette Position

The position of the glass within the filament will also affect the time it takes to pull a pipette. When using a trough filament, the glass should be about 0.5mm above the bottom of the filament and centered front to back. In the case of a box filament, the glass should be in the center of the filament. Filament positioning is covered in the next section of this manual "Heating Filaments".

3.9.2 Regulating the Length and Tip Size of a Sharp Pipette

3.9.2.1 HEAT

Higher HEAT settings will give longer and finer tips. A HEAT value equal to the Ramp Test value plus 10 units will generally give a very fine tip.

NOTE: At high HEAT settings (filament white-hot), the filament life is greatly reduced. Initially, use a setting equal to the ramp value plus 5: electrode length is controlled by air pressure adjustments. If this is insufficient, a wider or more narrow filament can be installed.

3.9.2.2 Filament Width

Filaments narrower than 2mm cannot form as fine a tip as the wider filaments. The tip size will decrease with increasing filament width until a width of 3mm is reached. Increasing the filament width beyond 3mm will produce longer and more gradual tapers (which may penetrate better in some cases). The tip, however, will not be any smaller.

3.9.2.3 Air Flow

In general, electrodes will not be formed if the air pressure is set too high. It is thus recommended that the pressure be set to standardized values of 500 for thick walled glass and 300 for thin walled tubing. However, as outlined above, under **Micropipette/microinjection needle fabrication**, the length of pipette tips can be controlled by varying air pressure. Furthermore, when making patch pipettes, if increasing TIME to its maximal setting of 255 does not provide enough cooling to produce tips with a short enough taper, then using the delay mode or increasing pressure above the standard values may be warranted.

3.9.2.4 DELAY Mode of Active Cooling

As discussed previously in this manual, switching from the TIME to the DELAY mode of active cooling may provide more precise and a wider range of control over the length (or taper) of a sharp pipette tip. The delay mode is often employed when using thick walled glass or for programs designed for the fabrication of pronuclear injection needles.

3.10 Fire Polishing

The P-1000 micropipette puller allows you to perform light to moderate fire polishing of pipette tips but does not have a provision for visualizing the pipette tip during the heating process. The extent of the heating required to attain the desired degree of polishing must be empirically established.

What distinguishes a program for polishing from other programs used to pull pipettes is the use of a Velocity value of 0 (zero). To program the instrument for the fire-polishing mode, try entering a program as follows:

HEAT	PULL	VELOCITY	TIME
Ramp value - 50	0	0	250

Pressure does not matter because the air supply is never activated in this mode.

When executed, this program will behave as follows: the Heat will turn on for the duration set by Time. Each Time unit is equivalent to 10 msec. Therefore, in the above program, the Heat will turn on for 2.5 seconds.

The procedure for polishing is as follows:

1. Pull a pair of pipettes with the desired pulling program. After the pipettes have been pulled, keep them clamped in the puller bars.
2. Reset the puller and select your polishing program (as above).
3. Manually push the puller bar (with the installed pipette) back towards the filament, and use the Fire Polish Spacer block described next. Using the adjusting screw on the top of the spacer, position the tip of the pipette just inside the edge of the filament.
4. Press Pull and the filament will heat up for 2.5 seconds, exerting a polish on the end of the pipette.

How much polishing occurs will be a function of the Heat value and the duration of that Heat as determined by the Time value. You may need to execute the program multiple times to achieve an appropriate polish. Experiment to determine how much Heat and Time are necessary for the degree of polishing required.

The most difficult part of this procedure is manually positioning each pipette back into the filament at the same relative position each time. The Fire Polishing Spacer allows you to consistently reposition the pipette within the filament. The T-shaped aluminum Spacer has an adjustable setscrew.

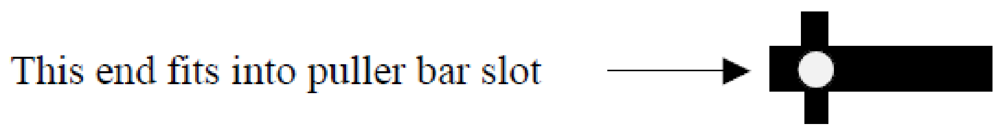


Figure 3-28. Side view of fire polishing spacer.

The Spacer/screw combination fits into the slot in the puller bar and fixes the puller bar position. You adjust the screw position to set the position of the puller bar (and thus the pipette tip). The more extended the screw is, the closer the pipette tip will be to the heating filament.

When you have finished polishing the pipette, remove the Spacer from the puller bar.

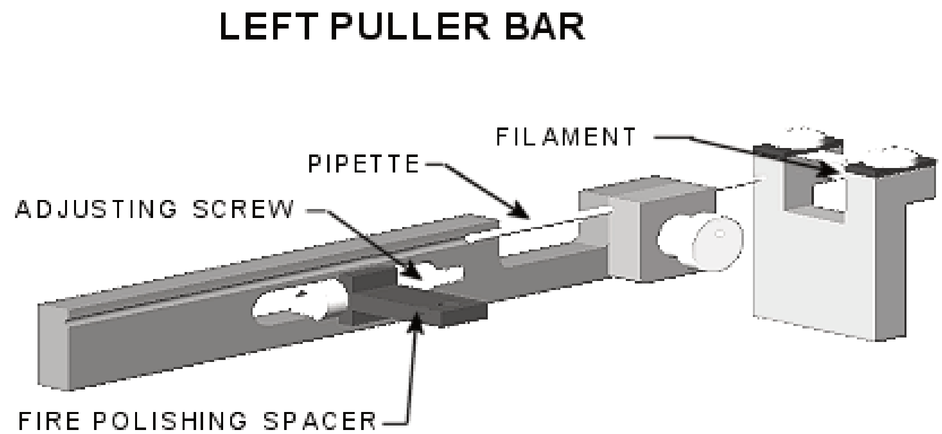


Figure 3-29. Fire-polish spacer in puller bar.

3.11 Heating Filaments

3.11.1 General Information

The pipette programs that you ultimately develop will largely depend on the type of heating filament installed in the puller and the glass that you use. Depending on your research application, there may be an optimum filament/glass combination that differs from the configuration with which the puller is currently set up. After reading through the following material, if you have questions about which filament to use for your application, contact our technical support staff.

3.11.2 Filament Positioning and Aligning

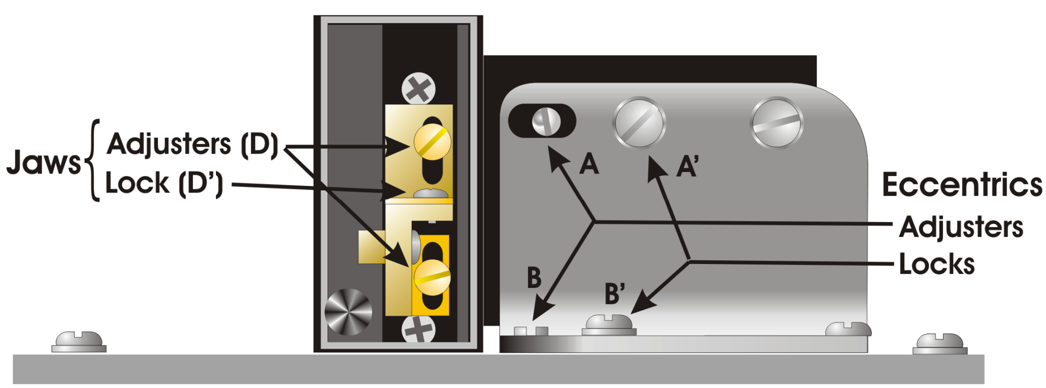


Figure 3-30. Filament alignment.

Adjust the filament position using the two eccentric chrome screws located on the silver or black angle piece that holds the filament assembly (A and B in **Figure 3-7**). First loosen the two locking screws (A' and B' in **Figure 3-7**) and then the filament can be moved in relation to the glass tubing by turning the appropriate eccentric chrome screw (A or B in **Figure 3-7**). See the Filament Replacement section of the Maintenance Chapter for a full description of this adjustment.

3.11.3 Box Filament

The box-shaped heating filament is recommended as the standard for most applications. The box filament heats the glass in a more symmetrical fashion than other filaments (such as the trough filament), so that the pipettes produced tend to be shorter and more straight and concentric. It delivers more heat to the glass resulting in faster heating without the necessity of increasing the temperature of the filament.

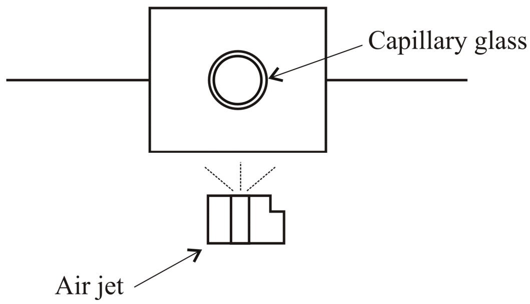


Figure 3-31. End view of box filament and glass.

Note: The Ramp Test value with a box filament will be higher than that with the trough filament, thus program HEAT values will be correspondingly higher in order to reach similar operating temperatures.

Box filaments are recommended for the following micropipettes:

- **Patch pipettes using thick-walled glass**
- **Microelectrodes used for slice preparations where long, parallel walls would aid penetration**
- **Microinjection needles for transgenic research**
- **Microdissection tools**
- **Thick or multi-barreled glass**
- **Aluminosilicate glass**

The box filament has two primary limitations.

First, it requires more current to heat to a given temperature than the same size trough filament. Thus, it is possible to use wider trough filaments without exceeding the maximum current capacity of the puller.

Second, the box configuration reduces the cooling effect of the air jet. For this reason, velocity settings often lowered.

3.11.3.1 <u>Positioning</u>

When using a box filament, the glass tubing should be centered vertically and horizontally (**Figure 3-30**). See section 3.11.2 for adjustments and alignment.

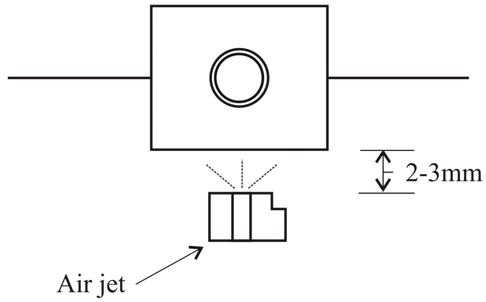


Figure 3-32. Box filament positioning.

3.11.3.2 Geometry

The box size you select should be approximately 0.5 to 1.5 mm larger than the outside diameter of the glass you are using. The width of the filament will depend on the research application. A good general-purpose box filament is the 2.5mm wide, 2.5mm high, and 2.5mm deep filament (**FB255B**). Special box filaments made to accommodate larger diameter glass or special pulling applications are available upon request.

Table 3-2. Box filament sizes.

Filament	Description	Glass O.D.
FB215B	2mm square x 1.5mm wide	1.0mm
FB220B	2mm square x 2.0mm wide	1.0mm
FB230B	2mm square x 3.0mm wide	1.0mm
FB255B (Standard)	**2.5mm square x 2.5mm wide**	**≤ 2.0mm**
FB245B	2.5mm square x 4.5mm wide	≤ 2.0mm
FB315B	3mm square x 1.5mm wide	≤ 1.5mm
FB320B	3mm square x 2.0mm wide	≤ 1.5mm
FB330B	3mm square x 3.0mm wide	≤ 2.0mm or 2-3 barrels

3.11.4 Trough Filament

The trough filament is a general-purpose filament. It is recommended for standard or thin wall glasses used for patch pipette fabrication, sharp electrodes with long tapers, and some types of microinjection needles.

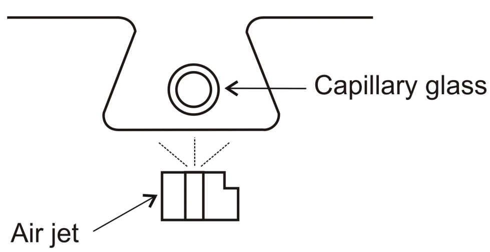


Figure 3-33. End view of trough filament and glass.

Note: The Ramp Test value with a trough filament will be lower than that with the box filament, thus program HEAT values will be correspondingly lower in order to reach similar operating temperatures.

3.11.4.1 Positioning

When using the trough filaments, the glass tubing should be positioned just above the bottom of the filament (approx. 0.5mm), and centered between the two sides (**Figure 3-33**). See section 3.11.2 for adjustments and alignment.

3.11.4.2 Geometry

The geometry of the trough filament is an important factor for proper heat application to the glass. Replacement trough filaments should have a profile similar to that illustrated in **Figure 3-33**, where the distance between the top corners (distance A) is approximately 2/3 the length of the bottom of the filament. This geometry will provide improved heat distribution to the top of the glass tubing. When replacing a filament, check the new filament geometry. If it differs appreciably from the ideal, you can easily modify it by grasping the bottom corners with non-serrated forceps and gently pushing on the horizontal 'wings'.

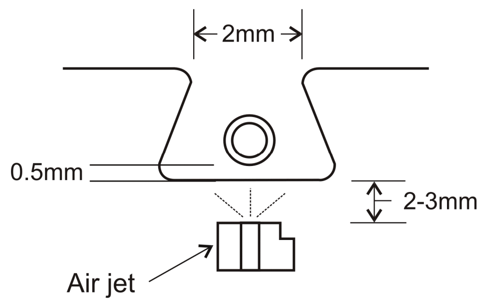


Figure 3-34. Trough filament positioning.

The trough filament you select depends upon the length of the taper that you want. Wider filaments for special purposes are available upon request.

Table 3-3. Trough filament sizes.

Filament	Description
FT315B	1.5mm wide trough
FT320B	2mm wide trough
FT330B (standard)	3mm wide trough
FT345B	4.5mm wide trough

4. MAINTENANCE

4.1 Cleaning

To maintain the P-1000 in optimal condition the vinyl dust cover that is shipped with the P-1000 should be used whenever the unit is turned off to protect the puller from dust and spills.

Occasionally clean the exterior and the base plate of the unit by wiping them with a dry cloth to remove dust and fine pieces of glass. Avoid contact with the filament.

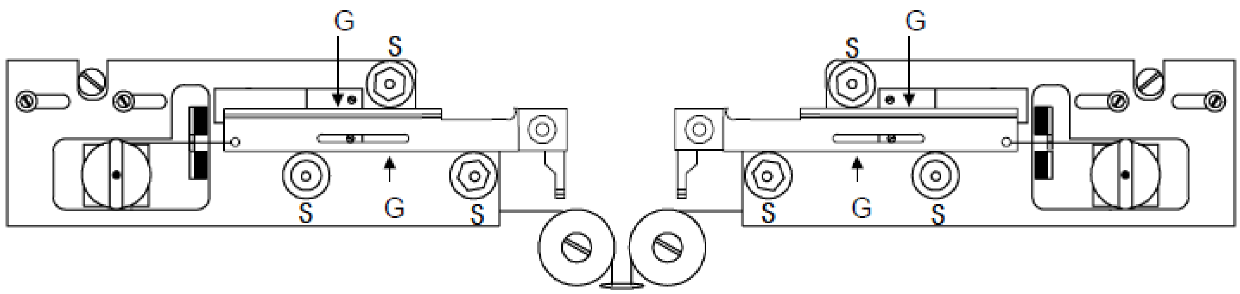


Figure 4-1. V-Groove bearings and puller bars.

Occasionally the V-groove bearings (**S in Figure 4-1**) and the edges of the pull bars that slide in their grooves (**G in Figure 4-1**) must be cleaned to maintain reproducibility from pull to pull. This should be done using a dry cotton swab.

⚠ ***CAUTION: DO NOT lubricate any components of the P-1000!***

4.2 Heating Filament Replacement

NOTE: See Sutter Instrument YouTube video "Installing a Filament (https://www.youtube.com/watch?v=cCsJsIZlzLw).

4.2.1 Filament Replacement (Step by Step)

1. Remove the chamber that encloses the heating filament (Remove the black thumbscrew at the lower left corner of the Plexiglas cover plate and pull the chamber straight off).
2. Loosen the two clamp screws (**D' in Figure 4-2, only one shown**) that hold the filament in place, and then slide out the old filament.
3. Slip in a new filament, center it over the air jet, and then retighten the two clamp screws.
4. Reinstall the chamber that encloses the heating filament and retighten the black thumbscrew (lower left corner of the Plexiglass cover plate).

4.2.2 Air Jet Position

The air jet should be from 2 to 3 mm below the center of the filament. If it not within this specification, then loosen the screw holding the air jet in place and reposition it.

4.2.3 Positioning the Filament in Relation to the Glass Capillary

The correct position of the glass capillary in each of the two filament types is shown above in the Heating Filament section. This positioning is critical in achieving the desired pipette tip size and shape; it will also almost certainly require adjustment after replacing a filament. To make this adjustment:

1. Carefully slide the glass to be used along the V-groove in the puller bar, and see where it is positioned relative to the filament.
2. Locate the aluminum angle plate to the right of the filament assembly and behind the right puller bar. Two chrome eccentric screws (**A** and **B** in **Figure 4-2**) in slots are mounted on this bracket, one located on the vertical face of the bracket and one on the horizontal face. Identify the flathead locking screws to the right of each chrome screw.

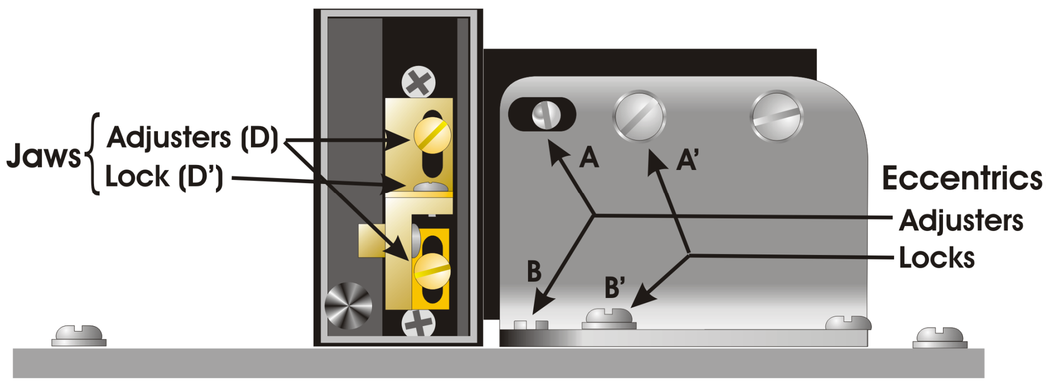


Figure 4-2. Filament alignment.

3. Loosen the locking screws (**A' and B'**)
4. Turn the eccentric chrome screw (A) on the vertical face to adjust the vertical position of the filament and the eccentric chrome screw (**B**) on the horizontal face to adjust the front-to-back position of the filament.
5. Tighten the locking screws (**A' and B'**)
6. If the vertical excursion available with the vertical cam screw is not enough to center the glass, you will need to reposition the upper and lower heater jaw assemblies by first loosening the brass screws holding the jaws to the black nylon (**D**). Reposition the jaws then retighten the brass screws and re-position the air jet.

Testing the Position: After positioning the filament it is important to determine if the filament is centered left-to-right over the air jet.

Run a RAMP TEST with your glass and the new filament.

Install a one-line program similar to the following:

HEAT	PULL	VELOCITY	TIME	PRESSURE
Ramp	70	70	250	500

This program is only being used to test pipette length. Pull a pair of pipettes. Remove the pipettes from the puller bars and hold them side by side as shown in the figure below. If the shanks of the pipettes vary in length, this is an indication that the filament is not centered left to right relative to the air jet, thus one pipette is “seeing” more cooling than the other. Loosen the filament clamping screws and move the filament very slightly towards the side that produced the shorter pipette. Then tighten up the clamps and try another pull. You may have to go through several iterations before you get it centered properly.

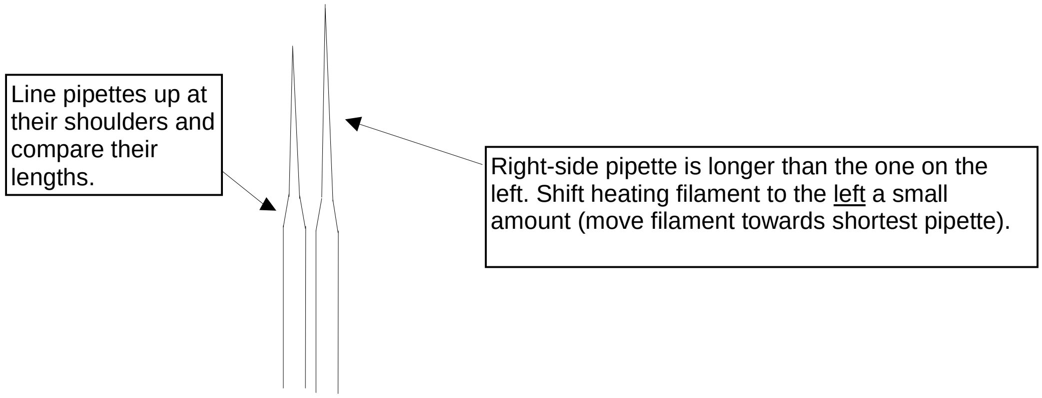


Figure 4-3. Micropipette shapes.

4.3 Pulley Adjustment

NOTE: Contacting Sutter Instrument Technical Support is highly recommended before performing a pulley adjustment.

The position of the two pulleys (**F in Figure 4-4A**) that guide the cables from the solenoid (**not shown**) to the puller bars (**G in Figure 4-4A**) is adjustable. The pulley position controls the tension of the cables. A difference between the tensions of the two cables can cause problems with pipette reproducibility and/or a disparity between the taper lengths of the pair of pulled pipettes (as illustrated in **Figure 4-4**). Taper length inequality is generally caused by the air jet not being aimed at the center of the filament, so to avoid unnecessary adjustments to the pulleys, be certain that the filament and air jet are correctly positioned before proceeding.

The pulley adjustment is made by moving one or both of the pulleys to equalize the tension on the two cables. There are two sets of stops in the system; the stops in the carrier slots against which the carriers rest (**M in Figure 4-4A**), and a stop to prevent the solenoid from being pulled out of its housing (**not shown**). The adjustment of the pulleys must be made so that the carriers will still come up against their stops while the solenoid is not against its stop. The two cables should not be under high tension when the carriers are against their stops (the position they would be in just before pulling an electrode).

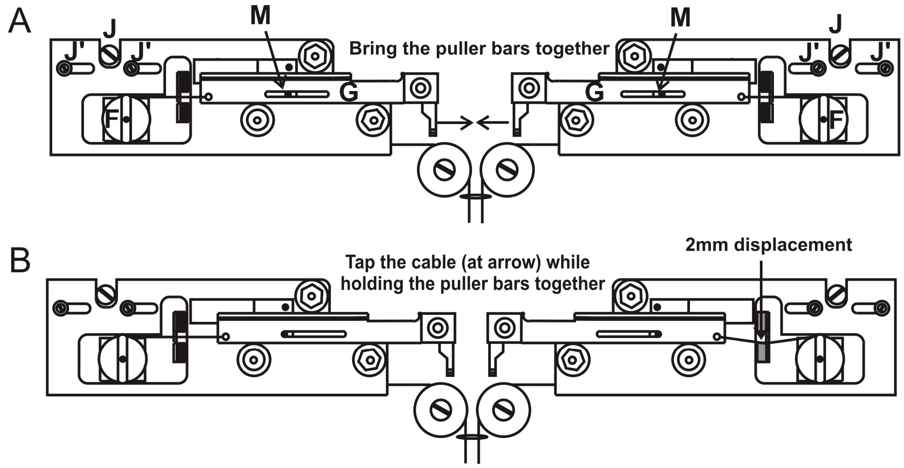


Figure 4-4. Pulley adjustment.

Holding the puller bars together with one hand, you should be able to press on either cable between the carrier and the pulley and feel about 2mm of deflection (**Figure 4-4B**) before the solenoid hits its stop. You will hear a clamping noise inside the chassis when the solenoid hits the stop. If the deflection is more or less, the pulley position should be changed. This is done by loosening the two screws above the pulley (**J' in Figure 4-4A**) and turning the chrome eccentric screws (**J in Figure 4-4A**) to move the pulley in small increments until the two cables are of equal tension. If the carrier no longer stops against its stop in the slot (**M in Figure 4-4A**), but stops against the cable, then the cam must be adjusted back until the carrier once more hits its stop. It is important that the carriers come up against their stops without significant tension on the cables. If there is too much tension, the initial pull will depend on how tightly you hold the finger stops when the glass is clamped in the carriers. If this happens, the electrodes will not be consistent from pull to pull.

4.4 Regeneration of Drierite Granules

The Indicating Drierite found in the canister at the right rear corner of the base plate on the P-1000 is a desiccant made of calcium sulfate (97%) and cobalt chloride (3%). This material is used to remove water vapor from the air-cooling supply system. The drierite granules become pink as they absorb moisture, eventually requiring that they be "regenerated" (dried).

4.4.1 Removing the Canister

Before proceeding, make sure the puller is off and unplug the power cord. To remove the canister from the -, first remove the plastic puller cover by loosening the three screws that hold it down. Next, slide the input (left) and output (right) air tubes off their white plastic connectors on the canister. Finally, the two black plastic clamps that secure the canister to the baseplate can be released by removing the screws at the base or, with older style clamps, forcing one half of the connector out of the other half at the point where they meet.

4.4.2 Replacing the Granules

Unscrew the end cap, being careful not to loose the black rubber-sealing ring that forms the airtight seal under the cap. With the cap off, the spring, the aluminum keeper, and the first filter can be removed exposing the Drierite. The exhausted granules can then be removed from the canister. **DO NOT REMOVE THE FAR FILTER AND ALUMINUM KEEPER.** The

Drierite should be spread evenly, one granule deep, on a tray and heated for one hour at about 200 degrees Celsius. The granules should then be cooled in a tight container before refilling the plastic canister. Drierite is not toxic and can be handled with few precautions. For more detailed safety information, please refer to the enclosed MATERIAL SAFETY DATA SHEET.

With the far keeper and filter in place, pour in the regenerated or new Drierite. Make sure the Drierite is well compacted set well. Next, insert the filter followed by the keeper and spring. Finally, make sure the O-ring is in the proper position in the cap and place a thin layer of vacuum grease on the O-ring. There is no need to over tighten the cover, but it should be possible to hear and feel it seating firmly against the rubber O-ring.

4.4.3 Reinstalling the Canister

Reinstall the canister on the puller baseplate with the cap to the left and the air tube connections to the front. The newer style plastic hold-downs can be tightened by reinstalling the front screws. The older style black plastic hold-downs slide inside one another and are pushed tight by hand to firmly hold the canister in place. **At this point, install the air input tube (larger tube, left-hand connector) and stop; do not install the output tube.** Plug in the puller and turn it on. The air pump will turn on and blow air through the canister. Allow this process to continue for several minutes. This procedure allows the purging of any dust or loose particles of Drierite produced during the recharging process. **It is critical that this dust not be blown into the output tube where it might clog either the air solenoid or the air jet.** After the purging process, you may connect the output tube and reinstall the puller cover.

If replacement is necessary, Indicating Drierite (8MESH with Indicator), manufactured by W.A. Hammond Drierite Co., Ltd. (Xenia, Ohio, USA), can be purchased from Sutter Instrument and most scientific supply distributors.

(This page intentionally blank.)

5. TROUBLESHOOTING

5.1 Controlling Pipette Tip Shapes

5.1.1 Problem: What type of glass should be used?

The type of glass and the wall ratio I.D. (inside diameter) to O.D. (outside diameter) are two of the most important variables in controlling tip size. For example, using borosilicate glass with an O.D. of 1.0mm and an I.D. of 0.50mm will give tips of 0.06 to 0.07mm as demonstrated in Program 0. Using the same settings, borosilicate glass 1.0mm O.D. and 0.78mm I.D. will form tips of 0.1 to 0.12mm. Aluminosilicate glass with an O.D. of 1.0mm and an I.D. of 0.68mm will form tips of 0.03 to 0.04mm again with the same settings.

In general, the thicker the wall in relation to the O.D. of the glass the finer the tip will be, and the thinner the wall the larger the tip will be. Thin walled glass will give the best results in most experiments as it will have the largest pore for a given tip size. This means it will have a lower resistance and will allow for easier injection of solutions. However, in many cases with small cells, the thin walled glass will not form tips fine enough to obtain good penetrations. In this case, heavier walled glass must be used.

5.1.2 Problem: The resistance of pulled pipettes is too low. How can a higher-resistance pipette be pulled?

The first point to note is that there is very little correlation between tip size and electrode resistance when pulling pipettes under 0.3mm. Most of the resistance of a microelectrode is in the shank of the electrode behind the tip. Electrode tips that are 0.1 mm in diameter can vary in resistance from 20MΩ to 1000MΩ depending on the length of the electrode and what is used for the filling solution. If the same solution is used then resistance may give an indication of how well the electrode will penetrate a cell as the electrode with the higher resistance will probably have a longer shank and a smaller cone angle at the tip. This combination will aid in the penetration of cells where the cell is not a surface cell.

5.1.3 Problem: How can tips be made even smaller?

1. The first thing to try in most cases is to increase the HEAT value. This will generally decrease the tip size but it will also give a longer shank. If the higher resistance is not a problem, this is generally the best solution. Continuing to increase the HEAT, however, is not the final answer as too high a HEAT can lead to larger tips. In general, with 1.0mm O.D.X 0.5mm I.D. borosilicate glass the finest tips will be formed when the glass pulls in 5 to 7 seconds after starting the pull.
2. If the electrode is now too long and results in a resistance too high to pass the necessary current, then the next step is to increase the pull strength. In general, a pull strength of 125 will give tips of less then 0.1mm. Increasing the pull to 250 will reduce tip size about 5-10%. We recommend a pull of about 150 in most cases.
3. The last major variable to adjust is the amount of cooling of the glass during the pull. If in the case of 1.0mm O.D. X 0.5mm I.D. borosilicate glass the pull takes place in 5-7 seconds, the tip size will not change with a change in the cooling air. The only change will be in the length of the shank. If however the HEAT is such that the pull takes place in more then 8 seconds, decreasing the cooling may somewhat decrease the tip size. Cooling

can be most effectively decreased in the P-1000 by decreasing air pressure, however a decreasing TIME may also be useful.

5.1.4 Problem: How can the size of a patch-pipette be increased?

1. The first thing to try is to reduce the HEAT on the last line of the program. Try dropping the HEAT 5 units at a time to see if this will increase the size of the tips.
2. If this does not work, increase the pressure in units of 50. The PULL should generally be set to 0 when pulling large tipped (1-10 mm) pipettes.
3. See also the Step 10 under "**Step-by-step patch programming**" in the **PARAMETER ADJUSTMENT** chapter and Chapter 1 in the Pipette Cookbook.

5.1.5 Problem: Patch-pipette tips vary in size from pull to pull.

This can happen when a pipette is formed in two or more loops. If the pipette is formed in three loops in one case and then on the next pull it forms in four loops the tips will not be the same. Adding one unit in the VELOCITY value will in most cases cause the pipette to be formed in three loops or subtracting 1 unit should cause the pipette to form in 4 loops. It is always good technique when a program is developed that produces a desired pipette, to try increasing and decreasing the VELOCITY value to be sure that you are in a stable region. The best procedure in developing a very reliable pipette program is to change the VELOCITY value both up and down until the number of cycles to pull the pipette changes. Then pick a value halfway between for the final VELOCITY value.

5.1.6 Problem: Difficulty making an injection pipette with a 0.5 – 1 mm tip that also has a very short final taper and tip (20 to 50 mm long). How can this be done?

(See "Bee Stinger" pipettes in Chapter 3 of the Pipette Cookbook.)

Try a program in which the first two lines of the program have a PULL value of 0, a VELOCITY value of 10 to 30, a TIME setting of 200 and use the ramp value for the HEAT (box filament). The third line should have the same HEAT value, a PULL value of 150, a VELOCITY of 30 and the TIME should be between 0 to 50 depending on the tip needed (values may vary depending on glass characteristics).

The idea behind this program is to reduce the size of the glass on the first two cycles and then on the third cycle we give a hard pull with the air turned off. Normally if the air is turned off a long wisp will result, but since we have greatly reduced the size of the glass and with a very hard pull the glass will tend to separate when it is about 1mm in diameter.

5.1.7 Problem: The electrodes are bent. How can they be made to pull straight?

This problem occurs most often when using the trough filament. Going to a box type of filament will produce straighter pipettes. The pipette's bend has no effect on its tip and should not cause problems unless when penetrating quite deeply into tissue and aiming at a certain site. Then the bend may lead the pipette to the wrong area. The box filament is not a complete improvement on the trough filament as the airflow is much less effective with the box filament, and you give up much of the length control that the cooling air gives with the trough filament.

5.1.8 Problem: One electrode is much longer than the other electrode.

This is caused by one of two things.

1. The filament may not be centered over the air jet. Follow the procedure "**Testing the position**" in the **Heating filament replacement** section of the Maintenance chapter.
2. If the filament is correctly centered, then the tension in the two cables that transmit the pulling force from the solenoid to the puller bars might not be equal. To check the tension and adjust if necessary, first contact Sutter Instrument to discuss the details and then follow the procedure "**Pulley Adjustment**" in the Maintenance chapter.

5.1.9 Problem: The shape and resistance of the pipette changes from pull to pull.

1. In most cases, this is due to **not** using the midpoint velocity when making a patch pipette or the program is unstable. See the Pipette Cookbook for recommended parameter settings for your application.
2. If the problem persists, then run the ramp test several times. If possible, use one long piece of glass and move the glass over after each ramp test. If the ramp values are +/- 4 units or less the problem may be with the glass. If the values are greater than +/- 4 units call Sutter Instruments.

5.2 System Operation/Function Problems

The P-1000 has many built-in automated test capabilities. When the system starts up, the hardware is tested for normal operation and error messages are generated upon detection of a failure. Additionally, automated testing occurs before each pull. Finally, a diagnostics menu screen is provided, allowing for diagnostic testing on demand.

5.2.1 Startup Problems

Table 5-1. Startup Problems Troubleshooting.

Problem on Start Up	Possible Causes and Solutions
Display is blank or locked up, and neither PULL nor RESET switch is illuminated	▪ Check power cord and wall AC power outlet. ▪ If the unit still does not work after verifying it is properly plugged in, remove the power cord and check the fuse. If the fuse has blown, it should be changed. However, if it blows a second time, contact Sutter Instrument Company Technical Support. ▪ If the fuse is still good, the unit is properly plugged in and it still does not work, a failure in components that are not serviceable by the user has likely occurred. Contact Sutter Instrument Company Technical Support.
Display is blank or locked up and either or both PULL and RESET switches are flashing on/off	A short exists in the controller's circuitry. The system must be returned to Sutter Instrument Company for repair.
Display is blank and both PULL and RESET switches are not illuminated or flashing on/off	Press RESET switch to restart the syste4m.

5.2.2 General Operational Problems

Table 5-2. General operational problem troubleshooting.

Problem	Possible Causes and Solutions
Displayed program values are not correct	1. Make sure that values were not changed by another user. Always write down the program values and the ramp-test value and keep them in a secure place. 2. If the values entered are not held when the power is turned off, a failure in components that are not serviceable by the user has likely occurred. Contact Sutter Instrument Company Technical Support.

5.2.3 On-Screen Emergency Reset Messages

Table 5-3. On-screen emergency reset messages.

Emergency Reset Messages	Possible Causes and Solutions
Circuit overheated Please wait for cool down to 70 C Heatsink temperature is 72 C	Indicates a serious fault in the system, requiring that it cool down. If the problem recurs after allowing sufficient time to cool down, the problem is likely due to a circuit fault, requiring repair at Sutter Instrument Company.

Emergency Reset Messages	Possible Causes and Solutions
Air Pump ran 5 minutes continuously. Please correct problem and RESET or Open Diagnostics	A massive air leak has occurred. Follow the instructions in the error message.
The Air Pump failed to reach pressure	A Serious air leak has occurred. (No leak test was possible.) A fault has occurred in the air pump or drive circuit.
The Air Valve failed to open	Electrical disconnection. A fault has occurred in the air valve or drive circuit.
There is a major airleak	The leak is probably serious enough to affect your pipettes, and if the leak causes air to flow through the Drierite canister, the Drierite will wear out prematurely. Trace the leak using the Help File assistant.
There is a fault in the Heat circuit.	This fault is due to a circuit problem – probably a failed FET (Field Effect Transistor). Run Diagnostics to determine the problem more precisely.
No filament current detected. The Heater Filament may be missing or broken. If not there is a Heat circuit fault.	The filament either is not installed or is burned out. If a working filament is installed, this fault is due to a circuit problem – probably a failed FET (Field Effect Transistor). Run Diagnostics to determine the problem more precisely.

5.2.4 On-Screen Error Messages (Edit/Pull Screen)

If Safe Heat is checked, Heat values are compared to Ramp. The following error messages can occur; follow the instructions in corresponding error message to correct the problem.

- Ramp = 0 - unable to Heat Check. Press Ramp or uncheck Safe Heat (at your own risk)
- Line x Heat > Ramp + 15%. Filament may burn out. Reduce Heat or run Ramp Test or uncheck Safe Heat (at your risk)
- Line x Heat < Ramp - 15%. Jaws may overheat. Increase Heat or run Ramp Test or uncheck Safe Heat (at your risk)

If Jaw PreHeat is checked, a Ramp value is needed. If a Ramp value greater than zero is not specified, the following error message appears.

Table 5-4. On-screen error message in Edit/Pull Screen when Ramp value is not specified.

Error Message	Possible Causes and Solutions
A Ramp value is needed to use PreHeat	If Jaw PreHeat is checked, a Ramp value greater than zero is needed.

When Pull is pressed, the program is checked for validity, and some hardware checks are performed. An erroneous line found in the current program or a failed hardware check can generate one of the following error messages.

Table 5-5. On-screen error messages in Edit/Pull screen when PULL is pressed.

Error Message	Possible Causes and Solutions
Line 1 Heat = 0	Obviously not a valid Program.
Lines with Heat = 0'. Please edit these lines.	These Program Lines are not valid.
Puller bars are separated. Reclamp glass. Then press PULL again	The puller Bars must be drawn together to the limit of travel. If they are correctly positioned, go to Menu/Diagnostics to check for a fault condition.
Heat current error. Is Filament present and intact? See Menu/Diagnostics if Filament is OK	Filament Heater current is not behaving correctly. To distinguish between a broken/missing Filament and a circuit problem, go to Menu/Diagnostics.
Failed to reach pressure	There may be an air leak or other problem. Go to Menu/Diagnostics to determine. Also, see Home/Help Files/ Air system diagnostics to trace leaks.
Failed to melt glass.	This message shows if the glass does not soften after 60 seconds of heating. The Filament may have burned out (go to Menu/Diagnostics) or the Heat value may be too low. Perform a Ramp Test and use Safe Heat mode.

Other error messages.

Table 5-6. Other on-screen error messages in Edit/Pull screen.

Error Message	Possible Causes and Solutions
Maximum 10 Program Lines	Too many lines in your Program.

5.3 Diagnostics

The P-1000's built-in diagnostics is started by selecting "Diagnostics" in the main menu screen.

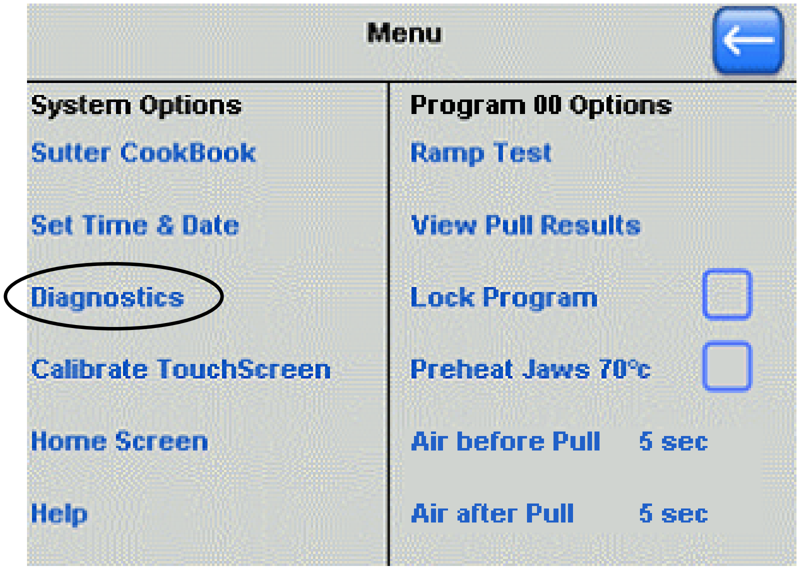


Figure 5-1. Starting Diagnostics from the Main Menu screen.

The Diagnostics screen provides the means by which each P-1000's hardware subsystem can be operated and tested independently.

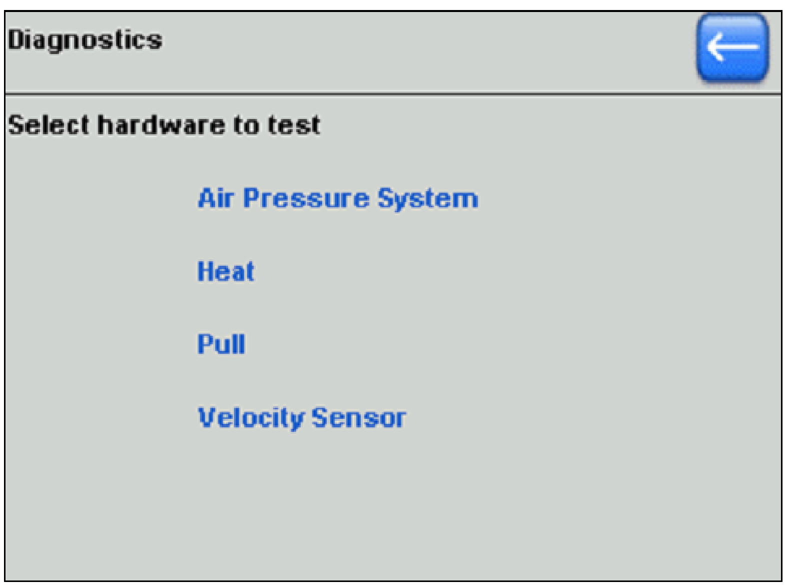


Figure 5-2. Diagnostics screen.

The Diagnostics screen can be invoked at any time should test and diagnostics be desired on any of the four hardware subsystems listed in the menu. These same diagnostics can be useful in troubleshooting problems while in telephone contact with Sutter Instrument Company Tech Support. The rest of this section covers the use of the Air, Heat, Pull, and Velocity Sensor subsystem diagnostics.

5.3.1 Air Pressure System

The Air Pressure System diagnostics menu shown in the following figure lists several diagnostic tests any one of which can be selected for specific tests.

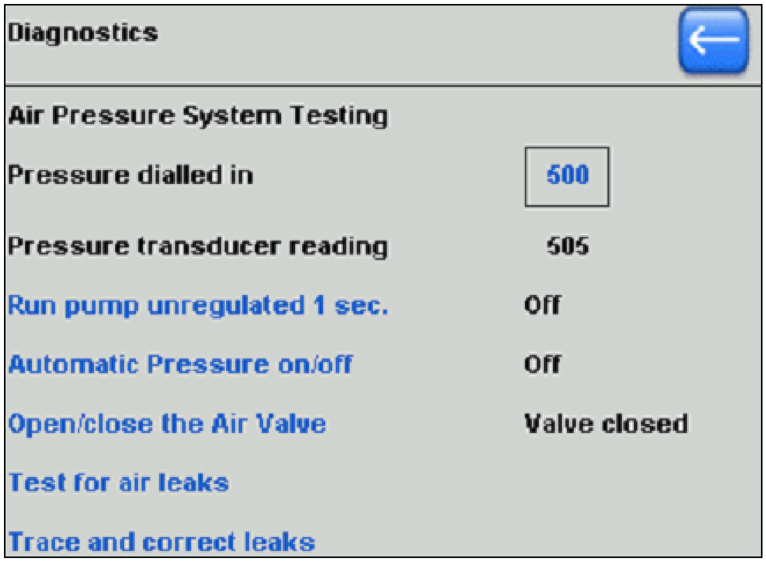


Figure 5-3. Air pressure system diagnostics screen.

5.3.1.1 Air Pressure Amount ("Pressure dialed in")

The amount of air pressure is shown in the "Pressure dialed in" field. This field is always highlighted so that different pressures can be selected using the front-panel Dial. Once the field contains the desired pressure value for testing, press ENTER to set that value.

5.3.1.2 Air Pressure Transducer Reading

The associated non-editable numeric field contains the pressure value from the transducer after a test has been made.

5.3.1.3 Air Pump Test ("Run the pump unregulated 1 sec.")

Run this test to verify that the air pump is working. While the test is running, the air pump will be audible, and if the test is successful, the pressure transducer reading will rise.

NOTE: The Air Pump Test can be run repeatedly to cause an increase in air pressure beyond its normal working range. No harm will result from doing this. However, the noise level coming from the air pump will become excessive.

5.3.1.4 Automatic Pressure On/Off

The Automatic Pressure test is a test of the air pump and the regulating circuitry. This test also checks for air leakage from the system.

5.3.1.5 Air Valve (“Open/close the Air Valve”)

This test verifies that the air valve opens correctly causing the displayed Pressure Transducer value to drop.

5.3.1.6 Test for air leaks

The air leak test consists of raising the pressure to 500 and then turning off the pump for 10 seconds while testing for a drop in pressure. During the 10 seconds that the pump is turned off, an error will be reported if the pressure drops more than 6 units.

5.3.1.7 Trace and correct leaks

This sequence of tests facilitates the detection and location of leaks in the air system, and provides guidelines on how to repair the leaks. Follow the instructions on the screen.

Tip: A good test for full flow through the air valve and air jet is as follows:

1. Set Pressure to 500.
2. Turn on both Automatic Pressure and Open the Air Valve.
3. The Pressure transducer reading should be 450 –500.

If the pressure is significantly lower than 450, the pump is weak and could affect the performance of your programs.

5.3.2 Heat Diagnostics

The diagnostics for the heating system is reached by the pressing Heat in the main diagnostics screen, whereupon the screen shown in the following figure is displayed.

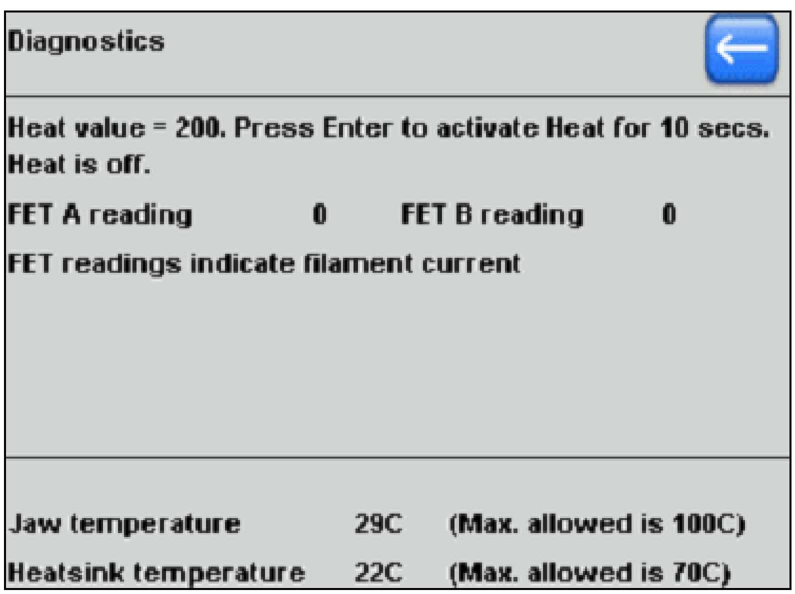


Figure 5-4. Heat system diagnostics screen.

To start the heat diagnostics, press Enter. The heating system is tested at a value of 200 for 10 seconds. The Heat current is controlled by two field effect transistors (FETs). FET A and FET B currents should both be equal to the Heat value of 200, within a few units. If they are unequal, there is circuit board fault. If they are both lower than the Heat setting, the Heat current is low. This can be due to a circuit board fault, but more often indicates a broken heat filament.

Fault conditions are reported in red after the test, as shown in the following figure.

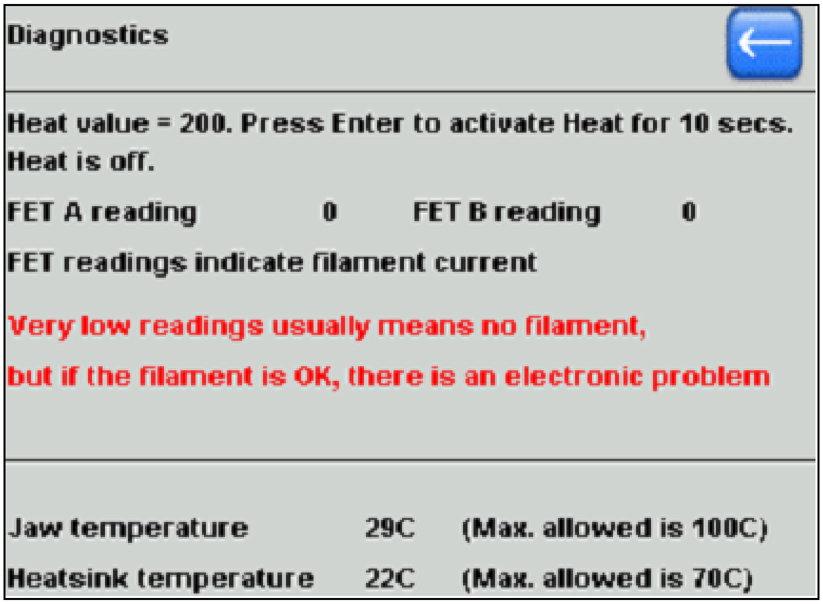


Figure 5-5. Heat system diagnostics error reporting.

The temperatures of the Puller Jaw and the internal Heatsink are continuously displayed. If Jaw Temperature exceeds the maximum allowed, the Heat will be automatically turned off to avoid damage to the nylon mounting block. If the internal heatsink temperature exceeds its maximum, a RESET will occur.

5.3.3 Pull Diagnostics

The Pull diagnostics screen is reached by selecting "Pull" in the main diagnostics screen. The pull diagnostics screen is shown in the following figure.

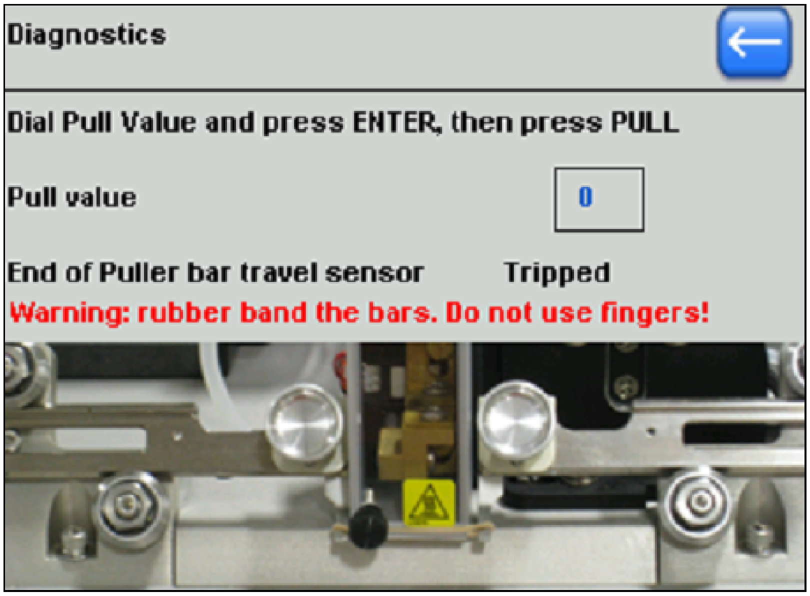


Figure 5-6. Pull diagnostics screen.

The Pull value is always highlighted for editing with the Dial. Press ENTER to set.

⚠ *WARNING: With a Dial setting of 255 (the highest possible), a violent separation of the Puller Bars occurs during a Pull, which can cause injury. Use a rubber band rather than your fingers to hold the bars together for any value over 100.*

⚠ *CAUTION: Never clamp a piece of glass to test a Pull. Something may get broken as a result.*

Press PULL to run the test. The End of Puller bar travel sensor shows "Tripped" when the bars are apart, not when they are fully together.

5.3.4 Velocity Diagnostics

The velocity diagnostics screen is reached by pressing "Velocity" in the main diagnostics screen. The Velocity diagnostics screen is shown in the following figure.

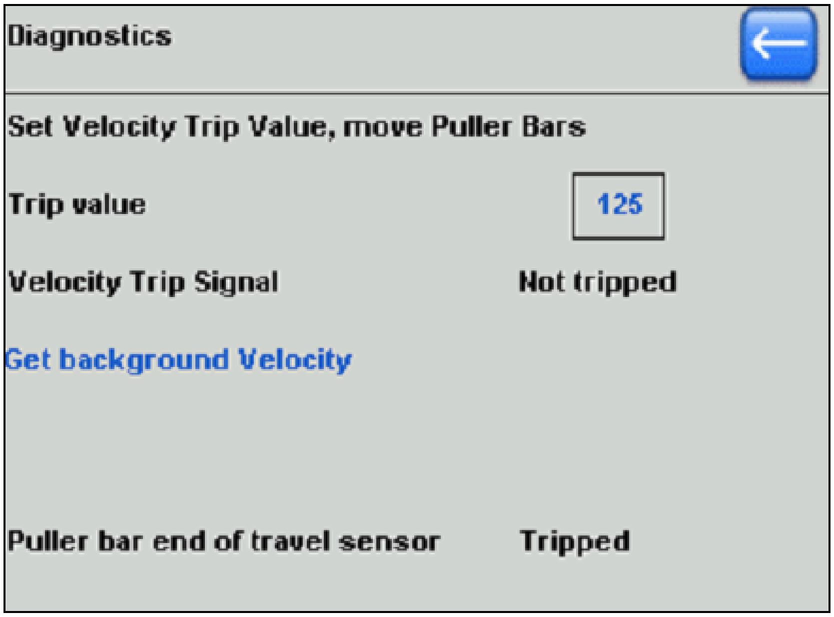


Figure 5-7. Velocity diagnostics screen.

In this screen, you can check the sensitivity of the velocity sensor and the operation of the optical sensor that detects when the glass has separated.

The Velocity value is always highlighted for editing with the Dial. Press ENTER to set.

When the Puller Bars move apart, a signal is generated that is dependent on the velocity of separation.

The Velocity setting is the threshold value at which the Trip occurs, as indicated on the screen.

A difference in sensitivity to movement at higher or lower settings can be noticed.

The Background Velocity serves to calibrate the true zero reading. It is typically 1-2 units. A higher number can indicate an electronic fault.

This calibration is done automatically at each pull.

The "Puller bar end of travel sensor" shows "Tripped" when the bars are apart and "Not Tripped" when they are fully together.

Adjust your Program Heat values to reflect the change in Ramp value.

5.4 Touchscreen Calibration

The touchscreen can drift out of alignment with the display over time. If ever the screen is so badly misaligned that the Touchscreen is not usable, recovery is still possible. Press RESET. When the Home Screen displays press PULL and the calibration routine will run.

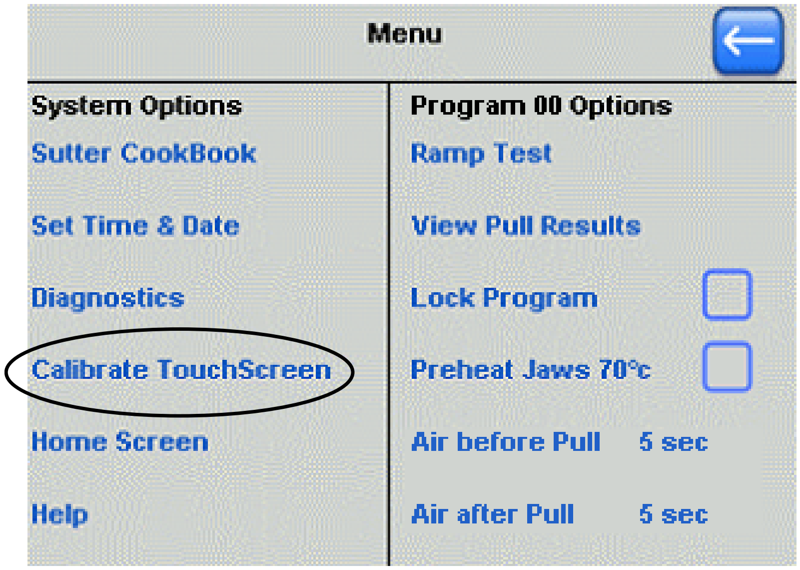


Figure 5-8. Starting Calibrate TouchScreen from the Main Menu screen.

The TouchScreen Calibration screen is started by pressing “Calibrate TouchScreen” in the Main Menu screen.

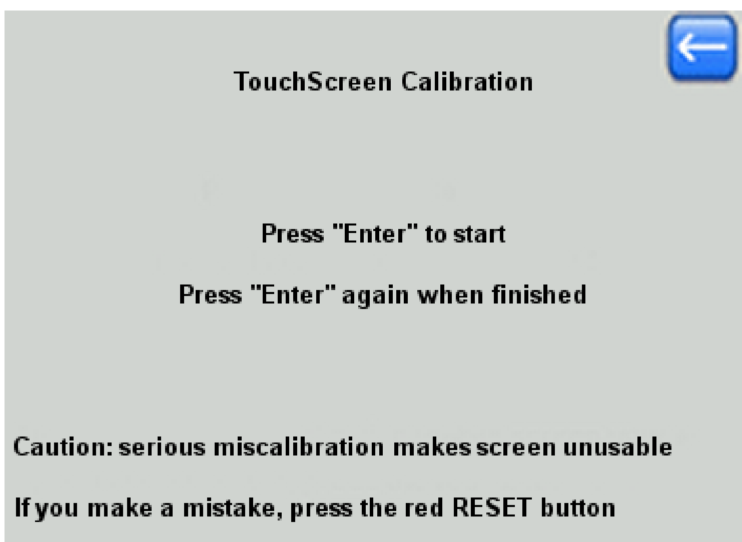


Figure 5-9. TouchScreen Calibration.

Follow the instructions on the TouchScreen Calibration screen to calibrate.

5.5 Technical Support

For further assistance, contact Sutter Instrument Technical Support at:

(415) 883-0128 **or** info@sutter.com

APPENDIX A. LIMITED WARRANTY

- Sutter Instrument Company, a division of Sutter Instrument Corporation, limits the warranty on this instrument to repair and replacement of defective components for two years from date of shipment, provided the instrument has been operated in accordance with the instructions outlined in this manual.
- Abuse, misuse, or unauthorized repairs will void this warranty.
- Warranty work will be performed only at the factory.
- The cost of shipment both ways is paid for by Sutter Instrument during the first three months this warranty is in effect, after which the cost is the responsibility of the customer.
- The limited warranty is as stated above and no implied or inferred liability for direct or consequential damages is intended.
- Consumables, PMTs, galvanometers, and Uniblitz®[1] shutters are exempt from this warranty.
- An extended warranty for up to three additional years can be purchased at the time of ordering, or until the original warranty expires. For pricing and other information, please contact Sutter Instrument.

[1] Uniblitz® is a registered trademark of Vincent Associates.

(This page intentionally blank.)

APPENDIX B. ACCESSORIES

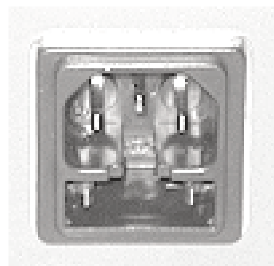
Filaments	Several sizes of of both Box and Trough filaments are available. Please refer to the Sutter Instrument Company's web site (www.sutter.com) for a list of part numbers.
FPS	Fire polishing spacer for P-97 and P-1000 pullers
GS	Glass stop (installs on either puller bar)
CTS	Ceramic tile for scoring glass (large tips 20-200 microns)
BX10	Pipette storage box (holds 10) 4 3/4 x 3 5/8 x 3/4 inches
BX20	Pipette storage box (holds 20) 7 x 3 5/8 x 3/4 inches

(This page intentionally blank.)

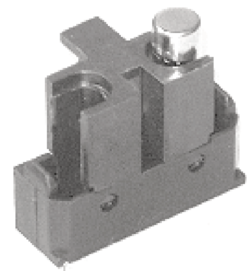
APPENDIX C. FUSE REPLACEMENT

In the event that the controller fails to power up when the power switch is turned on, check the line power fuse to see if it has blown. The fuse is located in the fuse holder on the power entry module on the back of the controller. To remove the fuse holder first unplug the power cord from the power entry module. This will reveal a slot just under the edge of the fuse holder. Use a screwdriver to pry the holder straight out of the power entry module.

Power entry module.



Fuse holder removed



Fuse holder (spare fuse not shown)

Figure 5-10. Power entry module and fuse holder.

The fuse that is readily visible in the fuse holder when you take it out is the one that is "active" when the holder is installed. A spare fuse is also stored within the fuse holder. Replace the active fuse with the spare and re-install the fuse holder and power cord. If the controller fails to power up with the new fuse installed, call Sutter Instrument technical support personnel for assistance.

⚠ **Replace fuse only with the same type and rating:**

Type:	**Medium Time Delay (or Time Lag), 5 x 20 mm glass tube, IEC 60127-2, RoHS compliant.**
Rating:	**T4A 250V (Time Delay, 4 Amps, 250 Volts)**
Examples:	**Bussmann S506-4-R or Littelfuse 218 004.P (or 218 004.HXP)**

(This page intentionally blank.)

APPENDIX D. TECHNICAL SPECIFICATIONS

Dimensions (H x W x D):	30 x 53 x 36 cm (12 x 21 x 14 in)
Weight:	18.53 kg (40.85 lbs)
Electrical:	
Mains voltage	100 - 240 V, 50/60 Hz
Power consumption	350W
Power cord	10A, 250V, with safety ground plug
Mains fuse (rear of cabinet)	Time delay (or time lag) 5 x 20 mm glass tube. For specific fuse ratings, refer to Appendix C.

(This page intentionally blank.)

APPENDIX E. DRIERITE MATERIAL SAFETY DATA SHEET

IDENTITY: INDICATING DRIERITE **DATE PREPARED** 1-3-96
DESCRIPTION: 1/16" TO 1/4" BLUE GRANULES

SECTION I

MANUFACTURER'S NAME:	W.A. HAMMOND DRIERITE CO. LTD.
ADDRESS:	P.0. BOX 460, 138 DAYTON AVE., XENIA, OH 45385
EMERGENCY PHONE NUMBER:	(513) 376-2927
INFORMATION PHONE NUMBER:	(513) 376-2927

SECTION II

INGREDIENTS

CHEMICAL IDENTITY	**%**	**OSHA PEL**	**ACGIH TLV**	**UNITS**	**C.A.S. #**
CALCIUM SULFATE	97	15	10	mg/M^3	7778-18-9
COBALT CHLORIDE	3	0.05*	0.05*	mg/M^3	7646-79-9

*(AS COBALT METAL)

HAZARDOUS MATERIALS IDENTIFICATION SYSTEM (HMIS)

HEALTH	**FLAMMABILITY**	**REACTIVITY**	**PROTECTIVE EQUIPMENT**
1	0	1	**E**

SECTION III
PHYSICAL/CHEMICAL CHARACTERISTICS

SPECIFIC GRAVITY:(H_2O=1):	1.87
SOLUBILITY IN WATER:	0.25 GRAMS PER LITER
MELTING POINT:	1450° C DECOMPOSES
APPEARANCE:	BLUE GRANULES; NO ODOR

SECTION IV
FIRE AND EXPLOSION HAZARD DATA

FLASH POINT:	NONE
EXTINGUISHING MEDIA:	NOT COMBUSTIBLE
SPECIAL FIREFIGHTING PROCEDURES:	NONE
UNUSUAL FIRE AND EXPLOSION HAZARDS:	NONE

SECTION V
REACTIVITY DATA

STABILITY:	STABLE
INCOMPATIBILITY (MATERIALS TO AVOID):	STRONG ACIDS
HAZARDOUS DECOMPOSITION BYPRODUCTS:	Cl_2 @ 318°C; SO_3 @ 1450°C
HAZARDOUS POLYMERIZATION:	WILL NOT OCCUR

SECTION VI
HEALTH HAZARD DATA

EYES: PARTICLES MAY CAUSE IRRITATION.
SKIN: THIS MATERIAL IS NOT TOXIC. MAY DRY OR IRRITATE SKIN
INHALATION: MAY CAUSE AN IRRITATION OF RESPIRATORY ORGANS OF SENSITIVE PERSONS RESULTING IN THE OBSTRUCTION OF AIRWAYS WITH SHORTNESS OF BREATH.
INGESTION: MAY CAUSE VOMITING, DIARRHEA AND SENSATION OF WARMTH
SIGNS AND SYMPTOMS OF OVER EXPOSURE: EYE, NOSE, THROAT, OR RESPIRATORY IRRITATION

CARCINOGENICITY OF INGREDIENTS:

MATERIAL	**IARC**	**NTP**	**OSHA**
CALCIUM SULFATE	NOT LISTED	NOT LISTED	NOT LISTED
COBALT CHLORIDE	YES*	NO	NO

*(COBALT & COBALT COMPOUNDS ARE CLASSIFIED AS GROUP 2B)

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE:

PRE-EXISTING UPPER RESPIRATORY AND LUNG DISEASE SUCH AS, BUT NOT LIMITED TO, BRONCHITIS, EMPHYSEMA & ASTHMA

EMERGENCY AND FIRST AID PROCEDURES:

EYES: FLUSH WITH WATER
DUST INHALATION: REMOVE TO FRESH AIR
SKIN: WASH WITH WATER
INGESTION: NONE KNOWN

SECTION VII
SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED:

SWEEP OR VACUUM MATERIAL INTO APPROPRIATE WASTE CONTAINER FOR DISPOSAL. AVOID DUSTING CONDITIONS.

WASTE DISPOSAL METHOD: THIS MATERIAL CAN BE DISPOSED OF AS AN INERT SOLID WASTE IN AN APPROVED LAND FILL OR BY OTHER PROCEDURES ACCEPTABLE UNDER FEDERAL, STATE AND LOCAL REGULATIONS.

PRECAUTIONS TO BE TAKEN IN HANDLING AND ST0RING:

KEEP CONTAINER CLOSED
STORE IN A COOL DRY PLACE
AVOID GENERATING DUST

SECTION VIII
CONTROL MEASURES

RESPIRATORY PROTECTION: NIOSH/OSHA APPROVED FOR DUST
VENTILATION: TO MEET TLV REQUIREMENTS
EYES: SAFETY GLASSES OR GOGGLES
OTHER PROTECTIVE EQUIPMENT: GLOVES OR PROTECTIVE CLOTHING ARE NOT USUALLY NECESSARY BUT MAY BE DESIRABLE IN SPECIFIC WORK SITUATIONS.

SECTION IX

REFERENCES
U.S. DEPARTMENT OF LABOR – OSHA FORM APPROVED OMB NO.1218 -0072.
OSHA HAZARD COMMUNICATION STANDARD 29 CFR 1910.1200
U. S. GYPSUM CO.

Although the information and recommendation set forth herein are presented in good faith and believed to be correct as of the date hereof, the W.A. Hammond DRIERITE Co. makes no representation as to the completeness or accuracy thereof. Information is supplied upon the condition that the person receiving same will make their own determination as to its suitability for their purpose prior to use. In no event will the W.A. Hammond DRIERITE Co. be responsible for damages of any nature whatsoever resulting from the use of or reliance upon information herein supplied. No representations or warranties, either expressed or implied, of merchantability, fitness for a particular purpose of or any other nature are made hereunder with respect to information or the product to which information refers.

INDEX

U

V

W

NOTES