OPERATION MANUAL

P-87

Flaming/Brown

Micropipette Puller

Rev. 0299c (20081016)

SUTTER INSTRUMENT

P-87

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Micropipette Puller

Operation Manual

(Rev. 0299c (20081016))

Sutter Instrument Company
One Digital Drive
Novato, CA 94949

voice: 415-883-0128 web: www.sutter.com
fax: 415-883-0572 email: info@sutter.com

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GENERAL INFORMATION

The P-87 can fabricate pipettes for use in intracellular recording, patch-clamping, microinjection 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 (0 &1) are already loaded in memory as is discussed in subsequent material.

The Model P-87 Flaming/Brown Micropipette Puller combines a proven pulling technology with programmability to produce a very versatile instrument. The pulling mechanism is derived from the P-77/P-80 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 multiplicity of pipettes on one device.

The P-87 is a 'velocity sensing' puller. This patented feature allows the puller to indirectly sense the viscosity of the glass, giving the P-87 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).

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 E-mail your queries to info@sutter.com.

SAFETY WARNING

- To prevent fire or shock hazard do not expose the unit to rain or moisture.
- To avoid electrical shock:

  Do not disassemble the unit. Refer servicing to qualified personnel.

  Always use the grounded power supply cord set provided to connect the unit to a grounded outlet (3-prong). This is required to protect you from injury in the event that an electrical hazard develops.
- 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.
- To avoid injuring your back or limbs, it is recommended that you do not attempt to lift this instrument by yourself. The P-87 Micropipette Puller weighs in excess of 18kg and should be moved by TWO people.

PRECAUTIONS

On Operation

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

- Operate the P-87 using 110V a.c., 60Hz or 220V a.c., 50Hz-line voltage.
- The P-87 is designed to be operated in a laboratory environment (pollution degree I).
- The P-87 is designed to connect to a standard laboratory power outlet (overvoltage category II).
- This unit was not designed to be operated at altitudes above 2000 meters nor was it tested for safety above 2000 meters.
- Operate only in a location where there is a free flow of fresh air on all sides. The fan draws air in through the vents on the sides and exhausts out both ends of the heat sink. NEVER ALLOW THE FREE FLOW OF AIR TO BE RESTRICTED.
- Since the P-87 is a microprocessor-controlled device, it should be accorded the same system wiring precautions as any 'computer type' system. If microprocessor based systems in the lab require line surge protection for proper operation, then the same protection should be provided for the P-87.
- Only use Sutter Instrument Company replacement heating filaments.

On 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 glassware recommended by Sutter Instrument Company in the following section of this manual.

GLASSWARE & HEATING FILAMENT SPECIFICATIONS

The P-87 micropipette puller is designed for use with aluminosilicate, borosilicate or other lower melting-point glass tubing or rod ranging from 1.0mm to 2.0mm in diameter. This instrument does not pull quartz glassware. Examples of the specific types and sizes of glassware that can be used with the P-87 are listed in the Sutter Instrument Company catalogue that was included with this instrument or can be viewed on Sutter Instrument Company's World Wide Web site at **www.sutter.com**. Any glassware with comparable technical specifications can be used with the P-87.

The type and size of glassware 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 glassware you would like to use. This instrument is designed to accommodate any of the Sutter Trough-type or Box-type filaments that are shown in the Sutter Catalogue. This selection of replacement filaments can also be viewed on Sutter Instrument Company's World Wide Web site at **www.sutter.com**. ONLY USE SUTTER-SUPPLIED REPLACEMENT HEATING FILAMENTS IN THIS INSTRUMENT! Instructions for replacing the Heating Filament can be found in the Maintenance section of this manual.

UNPACKING

Make certain that you have received all of the following items in the P-87 shipping box:

- P-87 micropipette puller
- Power cord
- Box of sample glass
- 4 Spare heating filaments
- Warranty registration
- Catalog

The Model P-87 is shipped to you in a prefabricated foam mold. Please take note of this method of packaging. Should it ever be necessary to ship the puller to another location, the same method of packaging should be employed. 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 as a result of such packing.

SETTING UP

Line power (Mains)

The power cord provided with the P-87 connects to the Power Entry Module located on the back of the unit (see diagram below). This Module also includes the Line Fuse and Voltage Selector Switch.

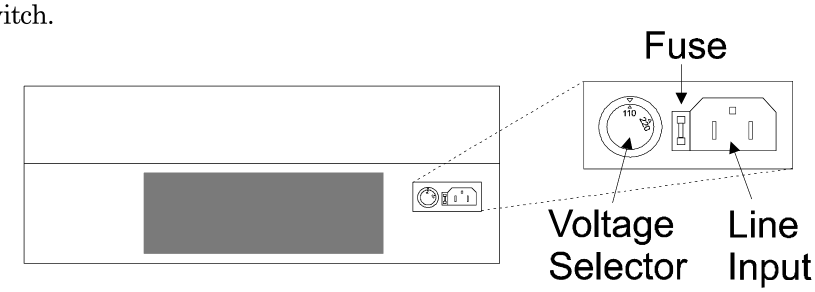

Figure 1. P-87 Cabinet (rear view).

Confirm that the Voltage Selector Switch on the Power Entry Module is set to the proper value (110V a.c. or 220V a.c.). **If it is not, turn the selector switch until the appropriate value is lined up with the indicator. Note that the Line fuse differs for the two different line voltages (see the Technical Specifications). If you have to change the voltage selector you may also have to replace the fuse, otherwise your protection from fire and electric shock may be compromised.**

Make certain that the Power Switch located on the left end of the P-87 cabinet is turned OFF.

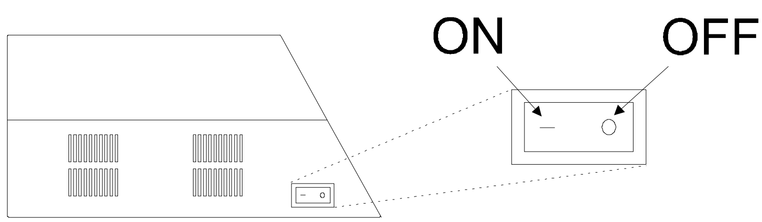

Figure 2. P-87 Cabinet (end view, left).

Plug the power cord provided with the P-87 into the Line Input socket on the Power Entry Module and then to a power source of the appropriate voltage and frequency.

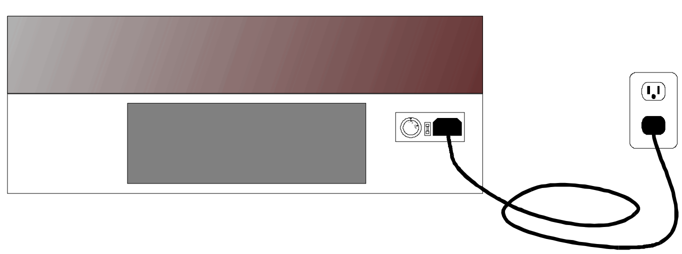

Figure 3. Power Connection.

FIRST TIME USE

While we realize that most new users of the P-87 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 because the user did not understand the relationship between the programmable parameter 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.

1. Make certain that the P-87 is plugged into the power outlet of the correct voltage and frequency.

2. Turn Power switch on left side of cabinet **ON**.

3. Load a piece of the supplied sample glass into puller as follows:

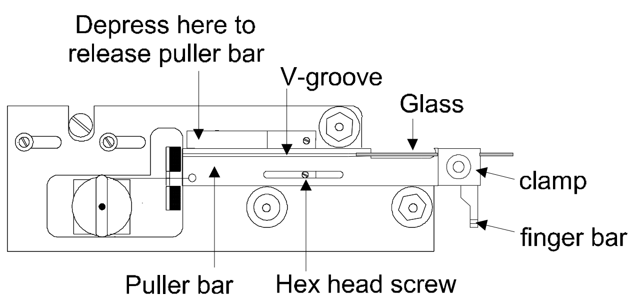

Figure 4. Left Puller Bar.

- Loosen clamping knob.

- Place glass in V-groove in puller bar, slide it beyond clamp about 2 cm and tighten knob.

- Depress the spring stop on each puller bar to release them from their catch position.

- Pull both bars towards each other using the finger bars. Hold bars in position using the thumb and index finger from one hand. The hex head screw should be touching the end of the slot in both puller bars.

- Loosen both clamping knobs, carefully slide glass through the holes in the side of the heater chamber and into V-groove of opposite puller bar.

- Tighten down clamping knobs.

4. Run a Ramp Test. This test is intended to help you determine a heat value that will melt your glass without burning out the heating filament. A Ramp Test should be run when using the puller for the first time; whenever you change the filament; whenever you change glass; before writing or editing a program.

   To run the Ramp Test
   - Enter any program number <0-9>
   - Press clear <CLR> to enter the control functions
   - Press <0> to not clear all parameter values

- Press <1> to run a RAMP TEST
- Install glass and press <PULL>.
- Record the Ramp test value, as it will be used to set Program HEAT

When a ramp test is executed, the following events take place:

- The puller increments the HEAT
- As the HEAT output begins to soften the glass, the puller bars will move apart.
- The heat is then turned off when a certain factory-set velocity is reached
- The Ramp Test value will be shown on the display.

To interrupt the RAMP TEST or reset the display after a ramp test, press <RESET>

5. Enter <0> to display Program 0. The cursor will be flashing on the **HEAT** value. Inspect the parameter values displayed. Program 0 should display the factory-installed values listed on the enclosed program sheet. Enter the **RAMP TEST** value you just ran for the **HEAT** parameter. Press the **PULL** key on the keypad. The heating filament should turn on and the glass should separate in less than 10 seconds.

6. Loosen the clamping knobs and remove the pipettes from the puller bars.

Unless otherwise stated on your program sheet, Program 0 is factory pre-programmed to pull a micropipette (tip diameter less than 0.1 µm) from 1.0mm x 0.5mm borosilicate glass. Program 1 is factory pre-programmed to pull a patch type pipette with a tip diameter of about 2µm. To try the patch pipette program (#1):

1. Press **RESET** to exit Program 0

2. Press **< 1 >** to enter into Program 1.

3. Enter the **RAMP TEST** value for the **HEAT** parameter. Press **PULL**.

FRONT PANEL

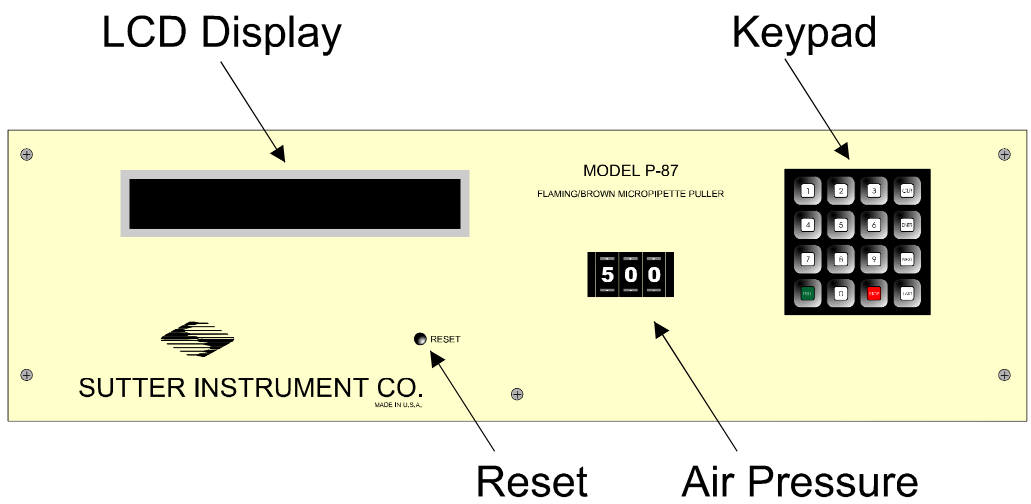

Figure 5. P-87 Front Panel.

Controls

LCD Display	Displays program parameters
Reset	Re-initializes the controller
Air Pressure	Sets the value of the air pressure during the active cooling phase of the pull cycle
Keypad	Used to program parameter values and execute programs
0-9	Used for choosing the desired program or control function, entering numeric values when programming and to make YES/NO (1/0) decisions
CLR	Used to delete programs or numeric values entered into those programs. This key is also used to access the RAMP TEST
ENTR	Used to enter new values
NEXT	Used to move to the next line in a program while editing
LAST	Used to move to the previous line in a program while editing
PULL	Initiates the execution of a program

STOP Aborts the execution of a program

Display

The P-87 has a two line LCD display for easy viewing. The following figure demonstrates what you will see after you have selected a program. A brief description of each parameter is provided below.

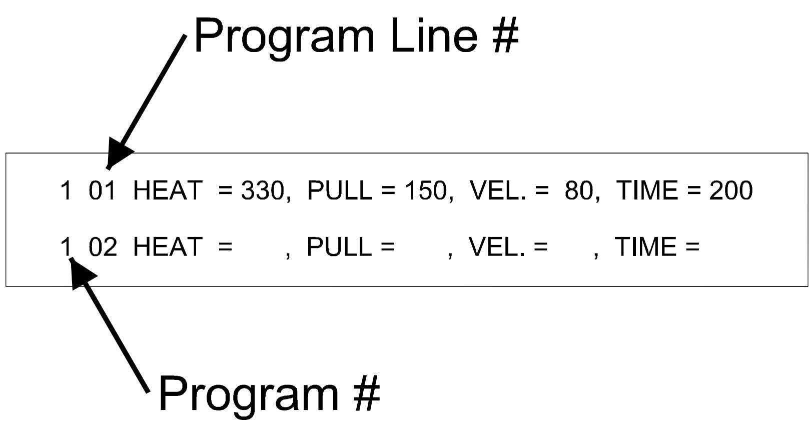

Figure 6. Program Display.

PROGRAM
(0-9)

A program consists of one or more cycles[1] which, when executed in sequence, will 'pull' the capillary glass inserted in the instrument. A program can be up to 16 cycles in length

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)

PULL controls the force of the hard pull. In general, the higher the PULL value, the smaller the pipette tip diameter and the longer the taper.

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[1] A **CYCLE** consists of four programmable parameters; **HEAT, PULL, VELOCITY** and **TIME**. A **CYCLE** is equivalent to one line of program code. The sequence of events during execution of a cycle is described in the PROGRAMS SECTION of this manual.

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 0-255)

TIME controls the length of time the cooling air is active. If VEL>0 then one unit of TIME represents 1/2ms. If VEL=0 then one unit of TIME represents 10ms. SEE THE PROGRAMS SECTION FOR A DISCUSSION OF THE SIGNIFICANCE OF TIME=0.

MECHANICAL DESCRIPTION (PULLER ANATOMY)

Some basic information

This section presents a basic mechanical description of the P-87, 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.

Air Cooling System

The Model P-87 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.

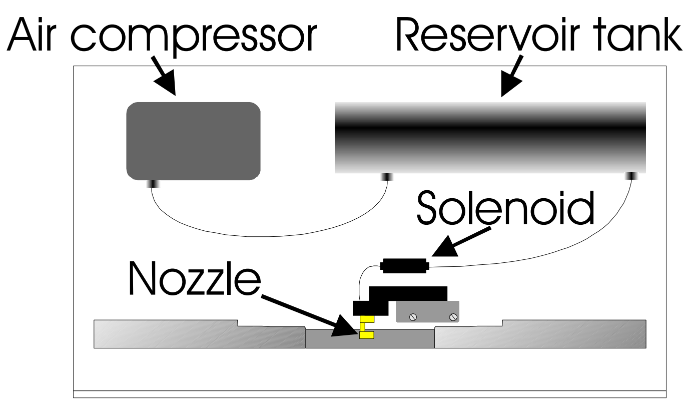

Figure 7. P-87 Base Plate (Top view, cover removed).

AIR COMPRESSOR (Error! Reference source not found.**)** Electronically controlled to pressurize the air reservoir tank

RESERVOIR TANK (Error! Reference source not found.**)** Low pressure vessel (filled with Drierite to remove moisture from the air flowing through the cylinder**)**

SOLENOID (Error! Reference source not found.**)** Regulates the flow of air from the air reservoir tank to the air nozzle in the filament area

NOZZLE (Error! Reference source not found.**)** Conducts air from the solenoid to the filament area. The nozzle should be located 2 to 3 millimeters below the filament and centered on it. The screw that secures the nozzle to the filament block can be loosened allowing the nozzle to move up and down.

Heating Assembly

The HEATING ASSEMBLY comprises the FILAMENT and the FILAMENT BLOCK ASSEMBLY. The FILAMENT BLOCK ASSEMBLY is discussed below. FILAMENTS are discussed in a separate section.

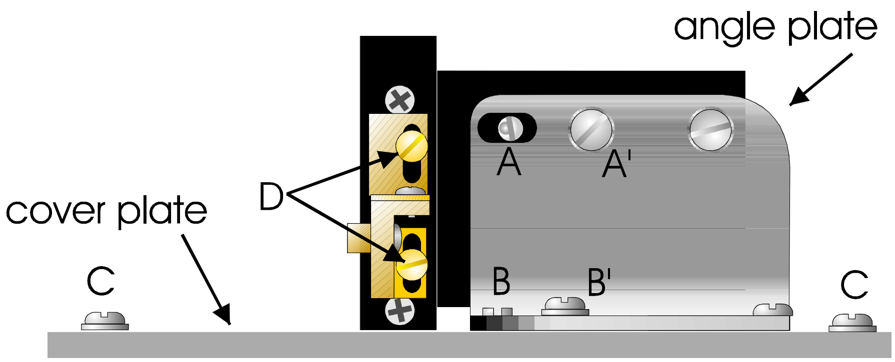

Figure 8. Filament Block Assembly.

FILAMENT BLOCK ASSEMBLY (Figure 8)

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 HEATER JAWS. Note that these jaws are slotted and may be moved up and down by loosening the screws **(D)** that secure them to the front of the filament block assembly. If the jaws are moved, make sure that the securing screws have been tightened; otherwise poor current flow can result in insufficient HEAT to melt the glass.

ANGLE PLATE (Figure 8)

The ANGLE PLATE secures the FILAMENT BLOCK ASSEMBLY to the COVER PLATE; it contains two important adjustments. Note the chrome-plated screws in slots at points **A** and **B** and the locking screws in slots at points **A'** and **B'**. The chrome-plated screws are 'eccentrics'; by rotating them 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.

COVER PLATE (Figure 8)

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'.

Upper Cable Pulley Assembly

This assembly guides the PULLING CABLES **(T in Figure 9)** from the PULLER BARS **(G in Figure 9)** 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 9)**, and contains a large eccentric adjustment screw **(J in Figure 9)**. This eccentric screw is used to adjust cable 'tension'. Its use is covered in the Maintenance Section.

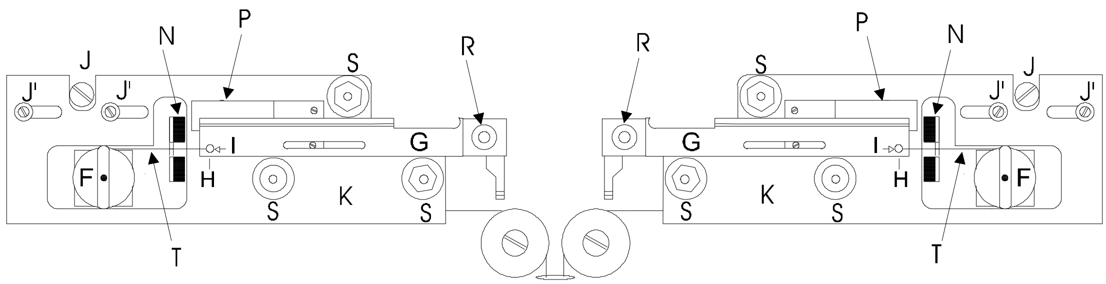

Figure 9. Upper Cable Pulley Assembly

PANELS, LEFT AND RIGHT (K in Figure 9)

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. Note the three socket-head cap screws that attach each PANEL to the top. These screws are used to align the PULLER BARS. Their adjustment, if necessary, is covered in the Maintenance Section.

BUMPERS (N in Figure 9)

The BUMPER stops the motion of its associated PULLER BAR, and prevents impact forces from breaking pipettes.

SPRING STOPS (P in Figure 9)

The SPRING STOPS are one-way catches that prevent pipette tip collision by catching the PULLER BARS as they rebound off the BUMPERS.

PULLER BARS (G in Figure 9)

This assembly consists of the puller bar, glass clamp, clamping knob and cable retaining screw. 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)**. 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.

V-BEARINGS (S in Figure 9)

These bearings are the guides for PULLER BAR motion. They are made of stainless steel and should **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 bushings are used to adjust position and ease of travel of the PULLER BARS (see Maintenance Section).

PULL CABLE (T in Figure 9)

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'.

Cabinet

BASEPLATE

The metal plate on which the mechanical assemblies are mounted.

BASE

The BASE includes the cabinet to which the BASEPLATE is mounted as well as the transformers and the circuit board contained within.

Electronics

The P-87 micropipette puller is controlled by a Z-80 microprocessor. Three digital-to-analog (D-A) converters control the HEAT, PULL and VELOCITY values. The HEAT power supply is a precision constant current switching unit which will vary less than 10 milliamperes with a plus or minus 10% change in the a.c. line current. The PULL supply is a constant current d.c. 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.

PROGRAMS

Program structure

The ultimate size and shape of a micropipette made using the P-87 is determined by the parameter values that are programmed by the user. Ten (10) separate programs can be saved for future use. Each program is structured as follows:

PROGRAM — Consists of one or more CYCLES which, when executed in sequence, will 'pull' the capillary glass inserted in the instrument. A program can be up to 16 cycles in length.

CYCLE — A CYCLE consists of four programmable parameters; **HEAT, PULL , VELOCITY** and **TIME**. A CYCLE is equivalent to one line of PROGRAM code.

Cycle parameters

Each of the four programmable parameters in a CYCLE are defined below:

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 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.

Special Condition: **VELOCITY = 0**

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 in a FIRE POLISH MODE.

TIME
(Range 0-255)

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

<u>**Special Condition**</u>: **TIME = 0**
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 microinjection or microperfusion.

Pull cycle

A typical PULL CYCLE in a PROGRAM is described below:

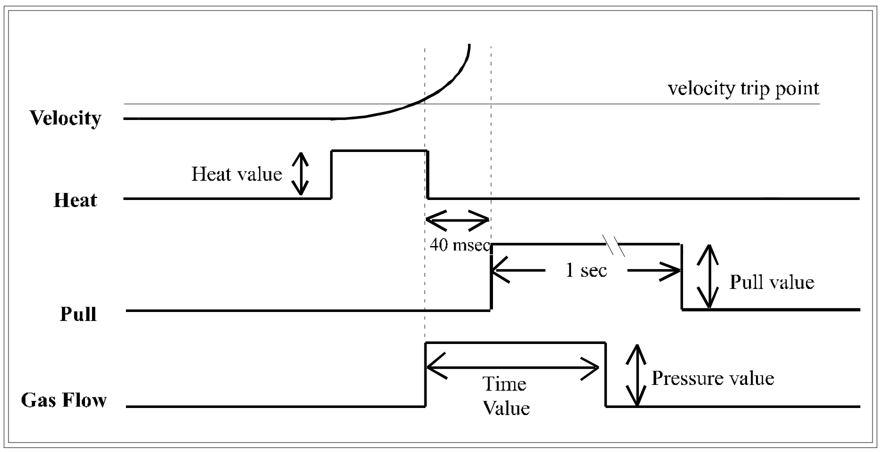

Figure 10. Pull Cycle.

1. The heat turns on.

2. The glass heats up 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 is turned on.

4. If TIME is >0 the hard pull (if any) is executed after a 40ms delay and the air is activated for the specified TIME (**Figure 10**).

Default Configuration

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

Table 1. Standard Factory Configuration

Heating Filament Installed	3mm trough (catalog # FT330B)
Glass used to program puller (sample sent with puller)	1.0mm O.D. x 0.5mm I.D. borosilicate, without internal filament (catalog #B100-50-10)
Factory installed programs (see enclosed program sheet)	0 - Micropipette (tip diameter less than 0.1 µm) 1 - Patch type pipette

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, in general, only affect your ability to control the geometry of the glass micropipette you are trying to fabricate. However, **the heating element can be destroyed by an excessive value for the HEAT parameter**. We recommend you refrain from executing a program until you have read through this section of the manual and have run the Ramp Test described herein.

Selecting a program

< 0 to 9 >
After applying power to the instrument, a "power on" reset will occur and the display will appear as follows:

```
WHICH PROGRAM DO YOU WISH TO USE? (0-9)
COPYRIGHT SUTTER INSTRUMENT CORP. 1991
```

Figure 11. Power on Display.

The P-87 has the capacity to store 10 programs (0 - 9). On the keypad, press **< 0 >** (factory installed micropipette program) or the number of another program you wish to execute. The display will appear as shown below in **Figure 12** (numerical values may vary). Please refer to the FRONT PANEL chapter and the beginning of this chapter for a full description of the PARAMETERS shown.

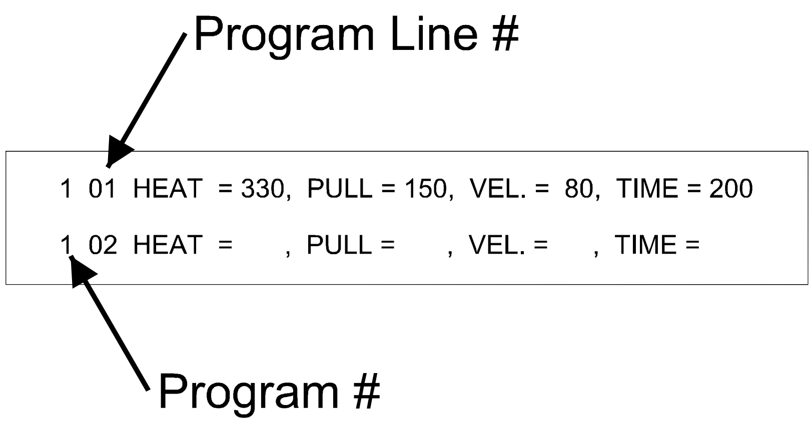

Figure 12. P-87 Program Display.

To select a different program,	Press **< RESET >** to bring up the sign-on prompt then press the number of the desired program (0 to 9).

Viewing a program

< NEXT > / < LAST >

The front panel display limits you to viewing only two adjacent lines of a PROGRAM at one time. When you first select a PROGRAM the display above will appear, showing the first two lines of the selected program.

- To view additional lines of the program press the **< NEXT >** key.
- To scroll back to previous lines press the **< LAST >** key.

Clearing a program from memory

< CLR >

To clear the values of a particular program from memory you can utilize the CLEAR function.

Pressing the **< CLR >** key gives you the following message:

```
DO YOU WISH TO CLEAR ALL VALUES FROM THE
PRESENT CYCLE TO THE END?  NO=0 YES=1
```

Figure 13. Clear Program Display.

Entering **< 1 >** will clear the PROGRAM from the line the cursor is on to the end of the PROGRAM. If the cursor is on Line 1, the entire program will be cleared from memory. If the cursor is on Line 2, Line 1 will be preserved, and lines 2-16 will be cleared.

Editing a program

Select a program number (e.g. 3). If there are already values entered, make sure that this program was not entered by another user of the puller. Unused program areas are usually cleared before a puller is shipped, but occasionally test program values are inadvertently left in memory. We recommend that unused programs be cleared before proceeding.

Entering a new program

After selecting or clearing a program area, the cursor will be blinking at the HEAT parameter on Line 1. To familiarize yourself with program entry, enter the following sample micropipette program:

HEAT	PULL	VELOCITY	TIME
300	150	100	150

Press the series of numbers < 300 > to enter the HEAT value of 300. The cursor will automatically tab over to the PULL parameter.

Enter < 150 > for PULL. The cursor will tab over to the VELOCITY parameter.

Enter < 90 > for VELOCITY. The cursor will remain there blinking. **Note that when less than three digits are entered, the cursor will not automatically tab to the next field.** You must press **< ENTR >** to enter the value and tab to the next field.

Enter < 150 > for TIME. The cursor will tab to the next field which is HEAT in Line 2.

Editing an existing program

To edit the value of a parameter(s) in a particular line, the cursor must be blinking in the field you want to edit. If necessary, press **< NEXT >** or **< LAST >** to scroll to the line you want to change. Press **< ENTR >** to tab the cursor to the field you want to change, and enter the new value. In our example above, to change the value of VELOCITY in Line 1 you first press **< LAST >** to scroll up to Line 1. Then press **< ENTR >** two times to tab over to the VELOCITY field. Note that the values for HEAT and PULL remain unchanged. Enter a new value (e.g. 90); if the value is two digits you must press **< ENTR >** to enter the value. The cursor will be positioned on Line 1 in the TIME field. To move to Line 2, press **< ENTR >** to "tab" over to the HEAT field in Line 2.

The program values above are only given to demonstrate data entry procedures. Useful program values to pull the pipette that you want will be discussed in the next chapter. In general, inappropriate settings will only affect your ability to control the geometry of the glass micropipette you are trying to fabricate. However, **the heating element can be destroyed by an excessive value for the HEAT parameter**. **Do not use HEAT settings greater than those found in programs 0 and 1 until you have run the RAMP TEST** described in the next section.

SOFTWARE CONTROL FUNCTIONS

The P-87 has two unique software CONTROL FUNCTIONS which allow you to run SELF-TEST procedures. These CONTROL FUNCTIONS are accessed through the **< CLR >** key. Pressing **< CLR >** gives you the following message:

```
DO YOU WISH TO CLEAR ALL VALUES FROM THE
PRESENT CYCLE TO THE END?  NO=0 YES=1
```

Figure 14. Access to Control Functions.

Entering **< 0 >** will provide you with access to the following P-87 CONTROL FUNCTIONS:

Table 2. Control Functions

Function #	Function Name	Description
1	RAMP TEST	Run the Ramp Test
2	MEMORY TEST	Test the integrity of the RAM

Select the desired CONTROL FUNCTION by pressing **< 1 >** for RAMP TEST and **< 2 >** for MEMORY TEST. The two tests are more completely described below.

RAMP TEST < 1 >

The HEAT value required to melt your glass is based on the characteristics of the heating filament that is installed and the 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. When executed, events in the RAMP TEST take place as follows:

1. The puller increments the HEAT at the rate of 500 milliamps per second.

2. The puller bars begin to move apart as the HEAT output begins to soften the glass.

3. The HEAT is turned off when a certain velocity (the value of which is stored in ROM) is achieved.

4. The RAMP TEST value shown on the display is the HEAT value that was required to reach the factory-set RAMP TEST velocity.

To interrupt the RAMP TEST or reset the display press RESET.

<u>Recommendations</u>

For Trough filaments:	Maximum program HEAT value(s) = Ramp value + 35 units. Recommended starting value = Ramp + 5 units.
For Box filaments:	Maximum Program HEAT value(s) = Ramp value + 30 units. Recommended starting value = Ramp value –20 units.

Additional guidelines for setting program HEAT values relative to RAMP TEST values are given in the Parameter Adjustment Section of this manual.

MEMORY TEST < 2 >

Performs a non-destructive test of the RAM. Press RESET to reset the system after performing this test.

PULLING PIPETTES

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 note the Ramp value reported. The HEAT settings in the factory-installed programs are typically set at Ramp value plus 5 units. If your Ramp test value differs from the factory Ramp value, adjust the HEAT in your programs to your Ramp value plus 5 units.

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 acquaint yourself with the pulling process.

1) Load the glass into the puller as described previously in the **FIRST TIME USE** chapter.

2) Press **< 0 >** on the keypad to view 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 plus 5 units.

4) Press the PULL key on the keypad. 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 line the program ended on, as follows:

```
THE PROGRAM LOOPED 01 TIMES.

THE LAST LINE USED WAS 01.
```

Figure 15. Pull Cycle Report.

5) Loosen the clamping knobs and remove the pipettes from the puller bars.

Unless otherwise stated on your program sheet, Program 0 is factory pre-programmed to pull a micropipette (tip diameter less than 0.1 μm) from 1.0mm x 0.5 mm borosilicate glass. It will pull the pipette in one heating cycle or 'loop'

A unique feature of the P-87 is its capability to loop through a program. This is demonstrated with the multicycle Program 1, which is factory pre-programmed to pull a patch type pipette with a tip diameter of about 2μm. Press RESET to exit Program 0, and then press **< 1 >** to enter into Program 1. The display for Program 1 should read similar to the following:

1 01 HEAT = 300, PULL = 0, VEL. = 50, TIME = 150
1 02 HEAT = , PULL = , VEL. = , TIME =

Figure 16. Sample Program.

Adjust the HEAT in the program to Ramp value plus 5 units. Load glass into the puller and press **< PULL >**. The heating filament should cycle on and off repeatedly. When the pull is complete, the display should read similar to the following:

THE PROGRAM LOOPED 04 TIMES.
THE LAST LINE USED WAS 01.

Figure 17. 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 50, 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 01 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.

Notes on Program Operation

There is always the possibility that the puller will be given a set of values which 'stall' its operation. An example might be where the HEAT value has not been set high enough to melt the glass, thus the glass can not 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.

All programs entered into memory (maximum of 10) remain there even after the power is turned off or the RESET switch is toggled. A special memory 'chip' that carries its own battery back-up will retain stored information for as long as ten years without power being applied to the instrument. Miracle that this is, it is strongly suggested that you keep a written record of your programs in case of unexpected difficulties.

PARAMETER ADJUSTMENT

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 through 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. Finally, as the amount of time required to pull a pipette is a fairly sensitive indicator of pipette characteristics, it is recommended that parameters are optimized with the aid of a handheld stopwatch to roughly gauge heating times.

Micropipette/microinjection needle fabrication

Consider the following programs using a 3mm trough filament:

Micropipette Program using 1.0mm x 0.5mm borosilicate tubing, pressure set at 500

HEAT	PULL	VELOCITY	TIME
Ramp value + 5	150	100	150

Microinjection Needle Program using 1.0mm x 0.75mm borosilicate tubing, pressure set at 300

HEAT	PULL	VELOCITY	TIME
Ramp value +5	60	80	200

HEAT

The HEAT setting will affect the length and tip size of the pipette. Generally speaking, higher HEAT settings tend to give longer and finer tips. For trough filaments, the recommended starting HEAT value is the ramp test value plus 5 units. For box filaments the recommended starting HEAT value is the ramp test value minus 20 units. The program listed above will typically have heat on for 5 to 8 seconds after the PULL key 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 needles you should select a HEAT value that melts the glass in about 7 seconds or longer.

PULL STRENGTH

Low values of PULL strength in the range of 40-75 will give larger tips appropriate for injection needles, while settings between 120-250 give smaller tips appropriate for micropipettes. The PULL strength can be set to any value desired with no danger of damaging the instrument.

VELOCITY (trip point)

The VELOCITY value determines the point at which the heat is turned off. VELOCITY reflects the speed at which the two carrier 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 (about 150) will produce equivalent results. This can be understood by referring to **Figure 10**. As the pull progresses, the speed of the carrier 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 120 for micropipettes or 50-80 for microinjection needles.

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.

TIME

The TIME parameter controls the length of time the cooling air is active (one unit of TIME is equivalent to 1/2msec). In order to produce effective cooling, the air must be supplied to the filament and glass during the time the tip is being formed. In order to guarantee that cooling occurs in this time frame, the start of the hard pull is begun after a fixed delay of 40msec from the termination of the heating cycle and the activation of active cooling. In most applications, the hard pull lasts several tens of milliseconds. Because of this fact, increasing cooling TIME values beyond a certain range (typically between 150 to 200) will have no effect. Values of TIME shorter than this same range 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 is a more effective means of controlling tip length (see below).

PRESSURE

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.

Note that increasing the pressure value to some higher number results in an immediate change in the pressure. A decrease in the pressure value will result in a pressure change at the time of the next pull. This delay in pressure change occurs because the system must first be purged of the air that is pressurized at the higher level. This will only occur prior to the execution of a program. Each time the PULL key is pressed the air solenoid is opened and air flows through the air jet until the pressure drops below the set value. The solenoid then closes and the compressor will pump until the correct pressure is attained. This pressurization routine is meant to fully cool the filament and assure that the pressure is precisely set.

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.

Patch pipette fabrication

Pipettes used for the electrophysiological recording technique of "patch clamping" are characterized by a short, stubby shank and relatively large diameter tips (> 0.7 μm). Programs which can fabricate a pipette with these characteristics generally differ from programs for micropipettes in three ways:

The trip velocity is lower.

The PULL strength is typically turned off.

More than one heating cycle is used.

The P-87 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 1.1 mm I.D. borosilicate glass using a 2.5mm box filament and with the pressure set at 500:

HEAT	PULL	VELOCITY	TIME
Ramp value – 20	0	70	200

A program sequentially executes each line of code and 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.

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.

PULL STRENGTH

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

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 first or second 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. However, too many cycles can lead to variability. Generally speaking, it is advisable to keep the number of heating cycles to 5 or less.

TIME

For patch pipette programs, it is advisable to keep the TIME between 200 and 250 to maximize the cooling of the glass. By contrast to micropipette pulling (see above), the TIME settings in the upper range of available values can have a pronounced effect on glass cooling. This additional cooling is effective due to the lack of a hard pull and the multiple cycle nature of the pulling process for patch pipettes.

PRESSURE

As with micropipette fabrication, the recommended pressure setting when using thick walled glass is 500 or greater. For thin walled glass the recommended setting is 300.

Note that increasing the pressure value to some higher number results in an immediate change in the pressure. A decrease in the pressure value will result in a pressure change at the time of the next pull. This delay in pressure change occurs because the system must first be purged of the air that is pressurized at the higher level. This will only occur prior to the execution of a program. Each time the PULL key is pressed the air solenoid is opened and air flows through the air jet until the pressure drops below the set value. The solenoid then closes and the compressor will pump until the correct pressure is attained. This pressurization routine is meant to fully cool the filament and assure that the pressure is precisely set.

Step-by-step patch pipette programming

1. 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.

2. Program one line of code as follows:

	HEAT	PULL	VELOCITY	TIME
for trough filament:	R + 5	0	*40	200
for box filament:	R – 20	0	*40	200

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

***The VELOCITY** value will need to be manipulated.

3. 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, 3 to 4 loops are typically all that is required. For thick walled glass, 3 to 5 loops are typically required.

4. Increase the VELOCITY in 5 to 10 unit increments and 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**.

5. Repeat step (4) only this time decrease the VELOCITY. As the VELOCITY **decreases**, the number of loops **increases**.

6. 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. For example: let's say that when you are 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.

7. The one line program just established may be sufficient for your application. However, changes made in a one-line program are amplified throughout the program and can produce 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:

8. 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 values

	HEAT	PULL	VELOCITY	TIME
loops 4 times	300	0	45	200

would be written into an equivalent 4 line program:

	HEAT	PULL	VELOCITY	TIME
line 1	300	0	45	200
line 2	300	0	45	200
line 3	300	0	45	200
line 4	300	0	45	200

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

10. 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 5 or 10 units. or increase or decrease VELOCITY in **last line** by 5 or 10 units or add a small amount of PULL (10 or 20) to **last line**.

Technical Tips

Regulating the time it takes to pull a sharp pipette

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 15 and 25 seconds after the start. Make corrections as outlined above for smaller tubing.

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".

Regulating the length and tip size of a sharp pipette

HEAT. Higher HEAT settings will give longer and finer tips. A HEAT value equal to the Ramp Test value plus 5 will generally give a very fine tip.

It should be noted that at high HEAT settings (filament white hot) the filament life is greatly reduced. It is suggested that a setting equal to the ramp value plus 5 should be used initially and electrode length should be controlled by air pressure adjustments. If this is insufficient, filament width can be varied.

Filament width. Filaments narrower than 2mm can not form as fine a tip as the wider filaments. Electrodes pulled using a 1.5mm filament will be very short and will have large tips.

Tips of 1-2µm can be formed using a 1.5mm filament with low filament temperatures and weak PULL strengths.

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 tips with a more gradual taper (which may penetrate better in some cases), however, the tip will not be any smaller.

Air flow. In general, electrodes will not be formed if the air pressure is set too high. It is thus recommended that the front panel pressure adjustment is 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 increasing pressure above the standard values may be warranted.

Fire Polishing

The P-87micropipette puller allows you to perform pseudo 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. To program the instrument for the fire- polishing mode, try entering a program as follows:

Heat= Ramp value Pull=0 Velocity=0 Time=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, or use the Fire Polish Spacer block described below. Try positioning 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. You will need to experimentally establish how much Heat and how much Time is necessary for the degree of polishing you require.

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 set screw protruding from it.

This end fits into puller bar slot

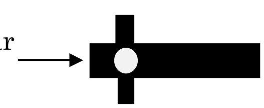

Figure 18. Side View of Fire Polishing Spacer.

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.

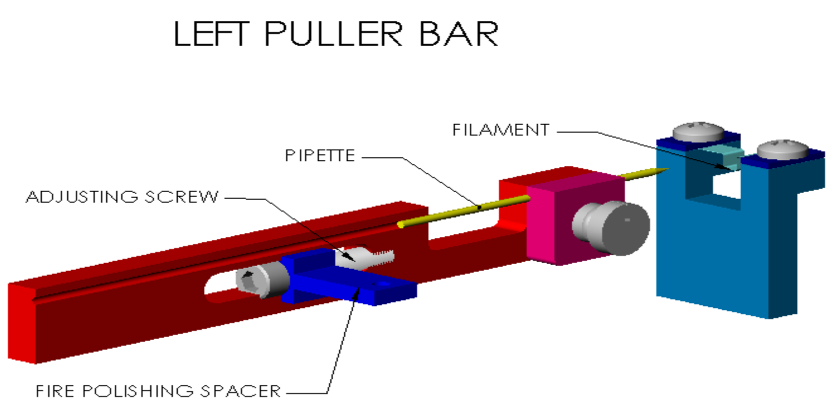

Figure 19. Fire Polish Spacer in Puller Bar.

HEATING FILAMENTS

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 which differs from the configuration the puller is currently set up with. After reading through the following material, if you have questions about which filament to use for your application, contact our technical support staff.

Trough Filament

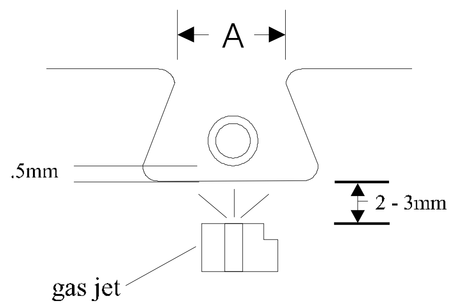

Figure 20. End View of Trough Filament and Glass.

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 short tapers, and some types of microinjection needles. The geometry of the trough allows the filament to cool rapidly during the cooling phase of the pulling process.

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 20)**. This position can be adjusted by using the two eccentric chrome screws located on the aluminum angle piece that holds the filament assembly **(A and B in Figure 23)**. First loosen the two locking screws **(A' and B' in Figure 23)** 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 23)**. See the Filament Replacement section of the Maintenance Chapter for a full description of this adjustment.

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 20**, 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'.

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. Trough Filament Sizes

Filament	Description
FT315B	1.5mm wide trough
FT320B	2mm wide trough
FT330B	**3mm wide trough**
FT345B	4.5mm wide trough

Box Filament

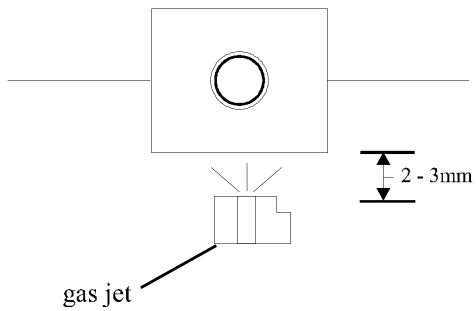

Figure 21. End View of Box Filament.

Another type of filament that can be used is the box type heater filament. The box filament heats the glass in a more symmetrical fashion than trough filaments, so that the pipettes produced tend to be more straight and concentric than those pulled with a trough filament. It delivers more heat to the glass resulting in faster heating without the necessity of increasing the temperature of the filament. **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:

Microelectrodes used for slice preparations where long, parallel walls would aid penetration

Microinjection needles for transgenic research

Microdissection tools

Thick or multi-barrelled 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 the box filament is not recommended when very short pipettes are to be formed (e.g. patch pipettes).

Positioning

When using a box filament, the glass tubing should be centered vertically and horizontally **(Figure)**. This position can be adjusted as described above for the trough filament. See the Filament Replacement section of the Maintenance Chapter for a full description of this adjustment.

Geometry

The box size you select should be approximately 1.0 to 1.5mm 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 4. 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	**2.5mm square x 2.5mm wide**	**≤1.5mm**
FB245B	2.5mm square x 4.5mm wide	≤1.5mm
FB315B	3mm square box x 1.5mm wide	≤2.0mm or 2-3 barrels
FB320B	3mm square box x 2.0mm wide	≤2.0mm or 2-3 barrels
FB330B	3mm square box x 3.0mm wide	≤2.0mm or 2-3 barrels

MAINTENANCE

Cleaning

To maintain the P-87 in optimal condition the vinyl dust cover that is shipped with the P-87 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.

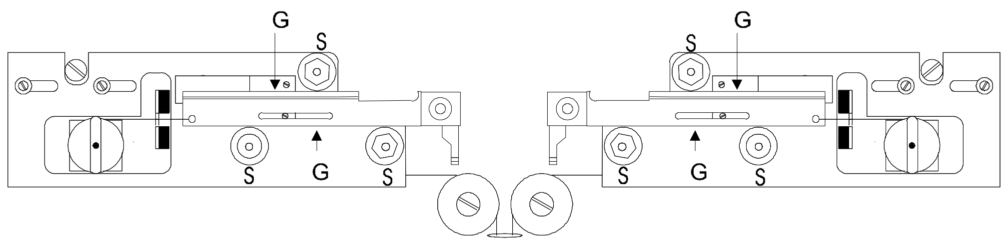

Figure 22. V-groove Bearings and Pull Bars.

Occasionally the V-groove pulleys **(S in Figure 22)** and the edges of the pull bars which slide in their grooves **(G in Figure 22)** must be cleaned to maintain reproducibility from pull to pull. This should be done using a dry cotton swab.

DO NOT lubricate any components of the P-87!

Heating filament replacement

Filament replacement.. The old heater filament can be easily removed by loosening the two clamp screws **(D' in Figure 23, only one shown)** that hold it in place. Slide out the old filament, slip in a new one and center it over the air jet. Then re-tighten the two clamp screws.

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

Positioning the glass tubing in relation to the filament. The correct position of the glass tubing in each of the two filament types is shown above in the Heating Filament Chapter. This positioning is critical to achieving the desired pipette tip size and shape and will 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 carrier 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 carrier bar. Two chrome screws **(A and B in Figure 23)** in slots are mounted on this bracket, one located on the vertical face of the bracket and one on the horizontal face. Identify the flat head locking screws to the right of each chrome screw.

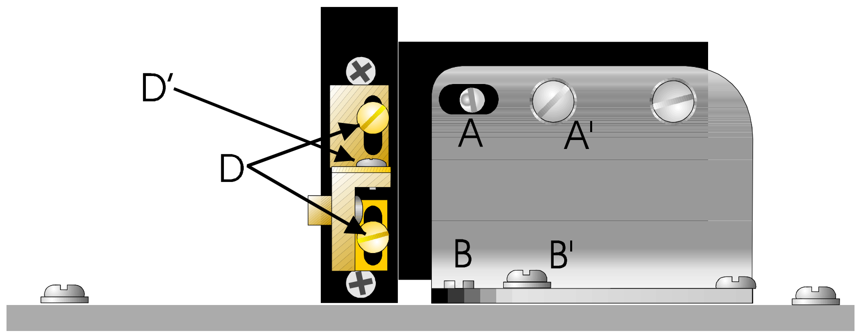

Figure 23. Filament Alignment.

3. Loosen the locking screws **(A' and B')**

4. Turn the chrome screw **(A)** on the vertical face to adjust the vertical position of the filament and the 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 isn't 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 Nozzle.

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. If you are unclear as to how to run the ramp test, please review that section of the Control Functions Chapter of this manual.

With the pressure set at 500, program a one line program similar to the following:

HEAT	PULL	VELOCITY	TIME
RAMP	120	100	150

This program is only being used to test pipette length. Pull a pair of pipettes. Remove the pipettes from the carrier bars and hold them side by side as shown in **Figure 24** 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.

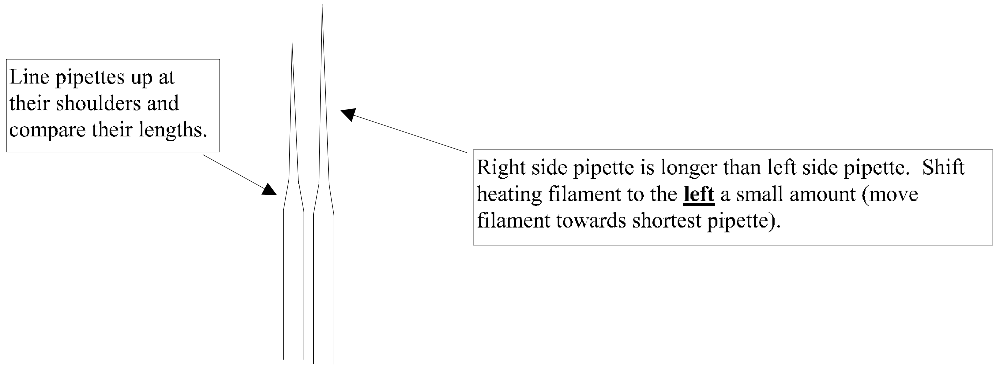

Figure 24. Micropipette Shapes.

Pulley Adjustment

The position of the two pulleys **(F in Figure 25A)** which carry the cables from the solenoid **(not shown)** to the carriers **(G in Figure 25A)** is adjustable. This adjustment should be made only if the two electrodes formed from one pull are of quite different lengths. This inequality is generally caused by the jet not being aimed at the center of the filament but may also be caused by unequal cable tensions. (To avoid unnecessary adjustments to the pulleys, be certain that the filament and air nozzle 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.

There are the stops in the carrier slots against which the carriers rest **(M in Figure 25A)**, 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).

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 25B)** before the solenoid hits its 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 25A)** and turning the chrome eccentric screws **(J in Figure 25A)** 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 25A)**, 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.

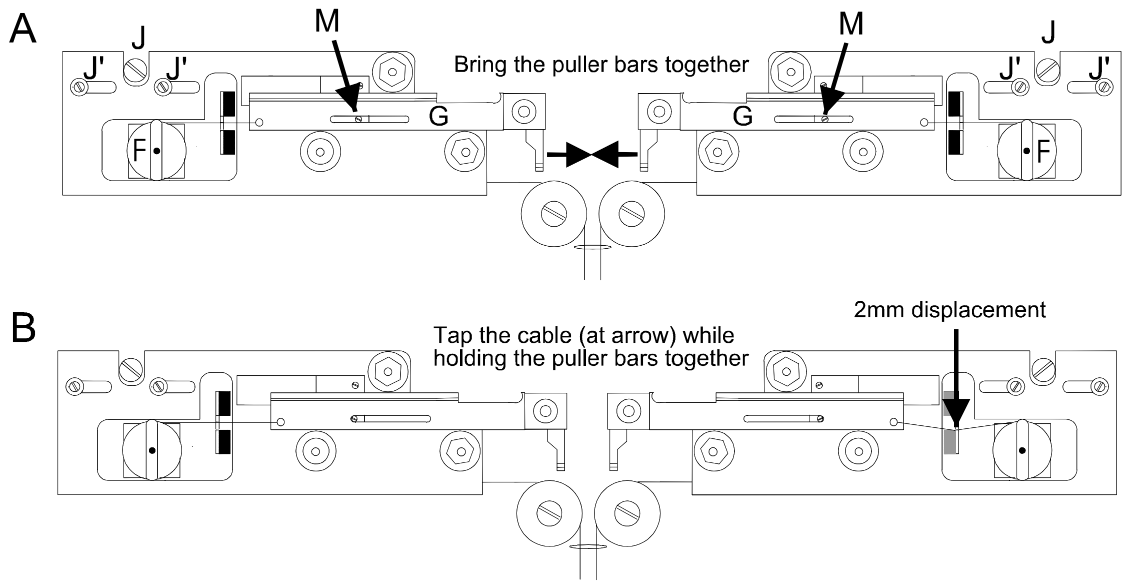

Figure 25. Pulley Adjustment.

Regeneration of Drierite granules

The Indicating Drierite found in the canister at the right rear corner of the base plate on the P-87 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. As it absorbs more moisture it becomes pink in color and must eventually be "regenerated" (dried).

Before proceeding, make sure the puller is off and unplug the power cord. To remove the canister from the P-87, you must first remove the 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 hold downs which secure the canister to the baseplate can be released by forcing one half of the connector out of the other half at the point where they meet. The canister can be removed.

Unscrew the aluminum 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, its associated aluminum keeper and the first filter can be removed exposing the Drierite. The exhausted granules can then be removed from the canister. The far filter can then also be removed. There is no reason to remove the far aluminum keeper. The Drierite should be spread evenly, one granule deep, on a tray and heated for one hour at about 200 degrees Celsius. <u>The granules should then be cooled in a tight container before refilling the plastic canister</u>. Filters should also be pre-dried at 100°C for about 30 minutes before assembly. 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.

The Drierite, filters and keepers are installed in the canister in the order they were removed. First install a filter against the keeper which was left in the canister. If this keeper was removed, slide it back in first and make sure that it lays flat against the plastic shoulders in the far end of the canister. With the far keeper and filter in place, pour in the regenerated Drierite. Next insert the second filter followed by the keeper attached to the spring. Finally, lay the rubber seal in the cover and screw on the cover being careful to keep the seal laid out flat as you tighten. There is no need to over tighten the cover, but it should be possible to hear and feel it seating firmly against the rubber seal.

Reinstall the canister on the puller baseplate with its cover to the left and the air tube connections to the front. The 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. This will allow air pump to come on and blow air through the canister and output into the room; allow this process to continue for several minutes. This procedure allows the purging of any dust or loose particles of Drierite that may have been 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 is manufactured by W.A. Hammond Drierite Co., Ltd. (Xenia, Ohio, USA) and can be purchased from most scientific supply distributors. 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.

TROUBLESHOOTING

Controlling Pipette Tip Shapes

Problem: WHAT GLASS SHOULD I USE?

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 0.07µm 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 0.12µm. Aluminosilicate glass with an O.D. of 1.0mm and an I.D. of 0.58mm will form tips of 0.03 to 0.04µm 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 wall 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 wall glass will not form tips fine enough to obtain good penetrations. In this case heavier wall glass must be used.

Problem: THE RESISTANCE OF MY PIPETTES IS TO LOW. HOW DO I PULL A HIGHER RESISTANCE PIPETTE?

The first point to note is that there is very little correlation between tip size and electrode resistance. Most of the resistance of a microelectrode is in the shank of the electrode behind the tip. Electrode tips which are 0.1 µm 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.

Problem: OK, BUT I STILL WANT A SMALLER TIP THAN I AM GETTING.

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 0.1µm. 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-87 by decreasing air pressure, however a decreasing TIME may also be useful.

Problem: HOW DO I INCREASE THE SIZE OF MY PATCH-PIPETTE?

1. The first thing to try is to reduce the HEAT. 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 μm) pipettes.

3. See also the step 10 under "**Step-by-step patch programming**" in the **PARAMETER ADJUSTMENT** chapter.

Problem: THE TIPS OF MY PATCH-PIPETTES 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.

Problem: I NEED TO FORM AN INJECTION PIPETTE WITH A 1μm TIP THAT IS 20 TO 50μm LONG. HOW DO I DO THIS?

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 a HEAT equal to the ramp value – 20 (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 1μm in diameter.

Problem: THE ELECTRODES ARE BENT. HOW DO I MAKE THEM PULL STRAIGHT?

This problem occurs most often when using the trough filament. Going to a box type of filament will produce straighter pipettes. The bend in the pipette has no effect on the pipette's tip and should cause no problems unless you are penetrating quite deep in the tissue and you are 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 air flow 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.

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. If it is not, 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 which transmit the pulling force from the solenoid to the puller bars is not equal. To check the tension and adjust if necessary, follow the procedure "**Pulley Adjustment**" in the Maintenance chapter.

Problem: THE SHAPE AND RESISTANCE OF THE PIPETTE CHANGES FROM PULL TO PULL.

1. In most cases this is due to one or both of the cables to the pipette carriers being set up too tight. If the cable is adjusted so that the carrier can't come against the stop in the slot in the center of the pipette carrier, then the initial pull tension will depend on how hard the carriers are squeezed together when the glass clamps are tightened. To adjust, see the pulley adjustment section.

2. A second possible cause of this problem is dirt on the carrier bars or bearings. In this case clean the carriers and bearings with a lint free tissue or cloth.

3. If the problem persists than 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.

Controller problems

Problem: THE FILAMENT DOES NOT LIGHT UP WHEN I PRESS PULL.

There are a number of possible reasons why this might happen.

1. Look and see if the filament has burned out. In some cases it may be necessary to loosen the screws holding the filament in place as a very fine break may be hard to see.

2. If the filament is OK, try running the ramp test and see what happens. If you have just changed the filament it is quite possible that the new filament needs a very different HEAT value than what you have been using. It is always a good idea to run the ramp test each time you change the filament.

3. If you run the ramp test and the HEAT value reaches 999 without the filament heating up check the tightness of the exposed connectors in the filament current pathway. These are the filament holding screws, the screws which hold the two brass jaws and the two nuts connecting the filament wires to the posts in back of the filament block.

4. If these connectors are tight then the problem is probably the power FETs on the heat sink.(Contact Sutter Instruments for further instructions.)

Problem: DISPLAY BLANK, FAN NOT ON.

1. Check power cord and wall a.c. outlet

2. If unit is properly plugged in and still does not work, remove the power cord and check the fuse. If the fuse has blown, a failure in components that are not serviceable by the user has likely occurred. Contact Sutter Instrument Company Technical Support.

3. 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.

Problem: DISPLAY SHOWS A ROW OF BLOCKS.

1. The microprocessor has failed to properly initialize the display. This problem can occur when the power has been turned off and then on again too rapidly. Press RESET and the display should show the proper power-up message. Always allow at least 5 seconds before turning the power back on. If the display still shows a row of blocks, a failure in components that are not serviceable by the user has likely occurred. Contact Sutter Instrument Company Technical Support.

Problem: 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.

Technical Support

For further assistance contact Sutter Instrument Technical Support at:

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

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.

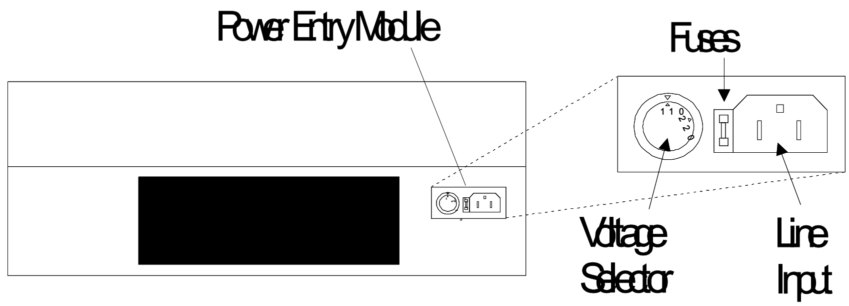

Figure 26. Power entry module.

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. It is concealed in a compartment as shown in **Figure 27**. To remove the spare fuse, press down on the end of the compartment and push it out of the other end. The old fuse can serve as a convenient tool for pushing the spare fuse compartment out. 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.

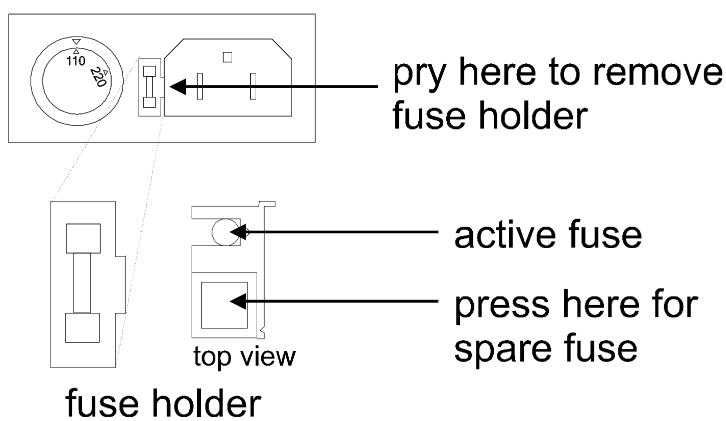

Figure 27. Fuse holder.

LIMITED WARRANTY

Sutter Instrument Company, a division of Sutter Instrument Corporation, limits the warranty on this instrument to repair or replacement of defective components for one year after the date of shipment, provided the instrument has been operated in accordance with the instructions outlined in the instruction manual.

Abuse, misuse or unauthorized repairs will void this warranty.

Limited warranty work will be performed only at the factory, the cost of shipment both ways to be borne by the user.

This instrument is designed to pull glass pipettes for use on animal tissues. It is not intended to be used and should not be used in human experimentation or applied to humans in any way.

The limited warranty is as stated above and no implied or inferred liability for direct or consequential damages is intended.

DISCLAIMER

The pipette puller Model P-87 is designed for the specific use of creating micropipettes and no other use is recommended.

This instrument creates items which should only be used in a laboratory environment for use on animal tissues. It is not intended to be used and should not 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.

TECHNICAL SPECIFICATIONS

Dimensions (H x W x D):	12” x 21” x 14” 30cm x 53cm 36cm
Weight:	60 lb. 27 kg
Electrical:	
Mains voltage	115 V, 60 Hz 230 V, 50 Hz
Maximum power consumption	370 VA
Mains fuse (rear of cabinet)	
at 115V	Type T (slow blow), 3A, 250V, GMC
at 230V	Type T (slow blow), 2A, 250V, GDC
Power cord	10A, 250V, with safety ground plug

APPENDIX A: 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.O. 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.

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