BIOCOMP

MODEL 152
PISTON GRADIENT
FRACTIONATOR

MODEL 153
GRADIENT STATION

MODEL 154
BIOHAZARD 4
FRACTIONATOR

MODEL 156/7
NANOFRAC
NANOSTATION

Software version 8.04

UV gradient profiling

Manual version 7.8

OPERATOR'S MANUAL

650 Churchill Row, Fredericton
New Brunswick, Canada E3B 1P6
Ph: (800)561-4221; (506)454-6410;
Fx: (506)455-6157; Email: dhc@biocompinstruments.com
Home Page: <http://www.biocompinstruments.com>

TABLE OF CONTENTS

BIOCOMP Model 152/153 Software Version 8.03

OPERATOR'S MANUAL

SECTION 1. INTRODUCTION

1.1 WARRANTY AND SERVICE

BioComp has a policy of complete customer satisfaction. If, during the first thirty (30) days, you are unhappy with the PISTON GRADIENT FRACTIONATOR (PGF), you may return it for a full refund.

BioComp warrants this instrument to be free of defects in workmanship for a period of one year from the date of receipt for all electronic and mechanical parts. Liability is limited to repairs or replacement of the unit at BioComp's discretion. This warranty is in lieu of all other warranties either express or implied.

Claims against this warranty must be made by first contacting BioComp **(phone (506) 454-6410)**. At this time the remedy will be determined. Units returned to BioComp without our knowledge and permission will not be accepted.

Claims for shortages or damage in transit must be reported to BioComp within ten (10) working days of the date of receipt. Such claims made after this period cannot be honored.

1.2 MAINTENANCE

Other than the tightening of loose screws and the removal of spilled gradient solutions, no user service is required. The PGF contains a computer which should not be tampered with. In the unlikely event of computer failure, contact BioComp and we will ship you a new printed circuit board and simple instructions for its installation.

1.3 ELECTRICAL SURGE PROTECTION

As with any computer, voltage spikes and power surges can severely damage the sensitive chips in this instrument. You are strongly encouraged to purchase a surge protector from your local computer or hardware store and plug the PGF into it. Since surges and spikes can occur at any time, it is also wise to turn the unit off when not in use to reduce your instrument's exposure to them.

1.4 GOODS DAMAGED IN TRANSIT

If the instrument or any of the accessories are damaged when you receive them, it is critical that you save the shipping carton(s) and contact us immediately. We will inform you of the return procedure and shipping addresses to put on the box. If the box is damaged to such an extent that returning the instrument in it would risk further damage, **save the box** for inspection by the shipper who will be notified to come and inspect it. Failure to adhere to these instructions will void any insurance claims we might seek and result in BioComp absorbing unnecessary expenses.

1.5 INSTRUMENT OVERVIEW

The principle of the Fractionator is this: a piston is forced into the centrifuge tube from above. It seals against the inside wall of the tube and forces the gradient out layer by layer by displacing it (Coombs, 1974, Anal. Biochem. **68:**95-1011.) The size of the fraction taken is determined by the length of the piston stroke. A key part of the evolution of the device has been in the shape of the piston face in contact with the gradient. It must somehow convert horizontal bands of particles into vertical tubes traveling up the outlet tubing. The current Trumpet Tip™ design doubles the peak height and halves the peak width compared with the ubiquitous bevelled needle (see the web page for the actual data).

The PGF also has an exquisitely sensitive band visualization system. T4 phage at $>10^8$/ml can be visualized with the naked eye. Ribosomal subunits are visible. This is possible for two reasons: light scattered by the particles from the bright spot light shining underneath the tube is seen against the black background of the tube holder. In addition, when the holder is filled with water before the tube is inserted, the outer surface of the tube essentially disappears since the refractive index of the solution and the tube are nearly the same. This lowers the background light scattering to such low levels that the faintest bands are visible. We also provide UV profiling at 254 and 280 nm for particles that do not scatter visible light.

Another key to sensitive fractionation is the ability to clean out the tubing between fractions with air or buffer. Often the top of a gradient contains large amounts of proteins, lipids or radioactivity that smear into lower fractions if the tubing is not rinsed. This problem has been eliminated by injecting air and/or buffer directly into the sample tubing at the point of gradient capture in the tip of the piston.

The software has been designed to be as simple as possible, but it will still take getting used to. The piston's downward movement at the start of the run is controlled by a dial. The further your turn it, the faster the piston moves downward. As you manually bring the tip of the piston into the tube, you will be asked to keep an eye on the outlet tubing to spot the liquid meniscus hitting your line on the tubing. This will become the "TOP" of every gradient, and fractionation can proceed with 10 micron accuracy below this point.. The rest is described below.

SECTION 2. SPECIFICATIONS

2.1 Physical Measurements

Shipping Weight: 50-52 lbs.
Dimensions: 19.25”W x 16.5”D x 20”H (piston down), X 27"(piston up)
Operating temperatures: Ambient (Cold room only if left turned on in the cold room and for 1 hr after return to room temperature to prevent condensation)
Input Power: 12 VDC, 2A, 50-60Hz.

2.2 Commercially available parts:

Stepper Motor: Hurst (Princeton, Indiana) TS -20 reduction cat no. SP3885
12V DC, 5A Power Supply: ASTEC LPS63
Air Pump: GAST (Benton Harbor, MI) #10D1-101-KGB
Water Pump: Greylor (Cape Coral, FL): #PQ-12DC
Liquid Crystal Display: Excel Technologies (Belle Meade, NJ) # LCM 2420 SFBL

2.3 Performance Characteristics

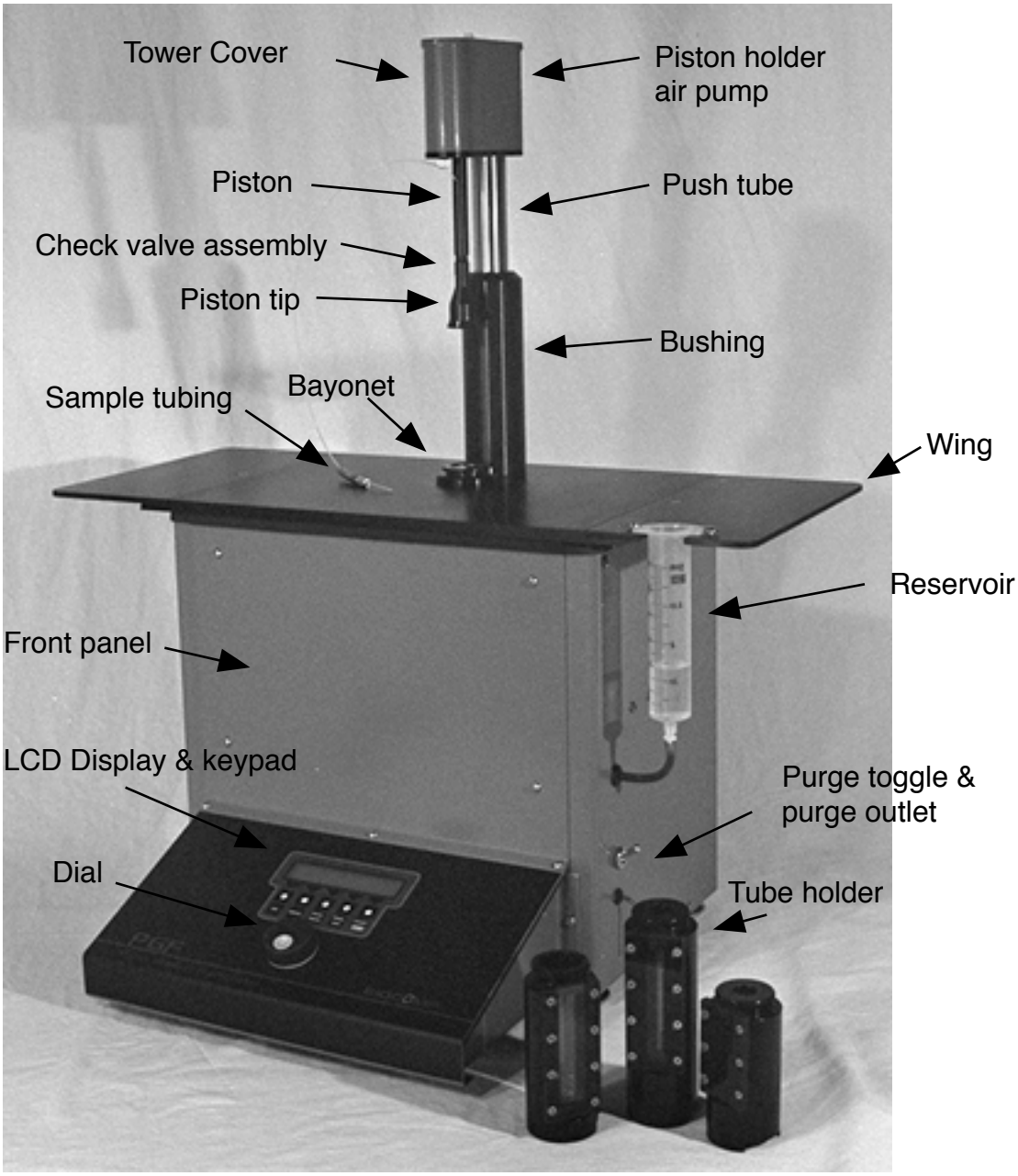
Piston Speed: 0.01-6.5 mm/sec down, 6.5 mm/sec upward
Fraction Size: 0.01mm - full tube length
Number of Fractions: 1-99 in each of 10 sections per gradient
Band Illumination System: 20W halogen spot with dichroic reflector
Rinse Capability (programmable in any order, up to 9 events between each fraction):

- Continuous air to section outflow with air bubbles during fractionation
- Air on demand or automatically between fractions: 15 msec to 9 sec
- Water/Buffer on demand or automatically: 15 msec to 9 sec
- Delay (a programmed interval between fractions): 15 msec to 9 sec
- Pause (an indefinite pause between fractions, broken by pressing START)
- Fraction Advance (a contact closure that signals a connected fraction collector to advance one fraction): 15 msec to 9 sec pulse

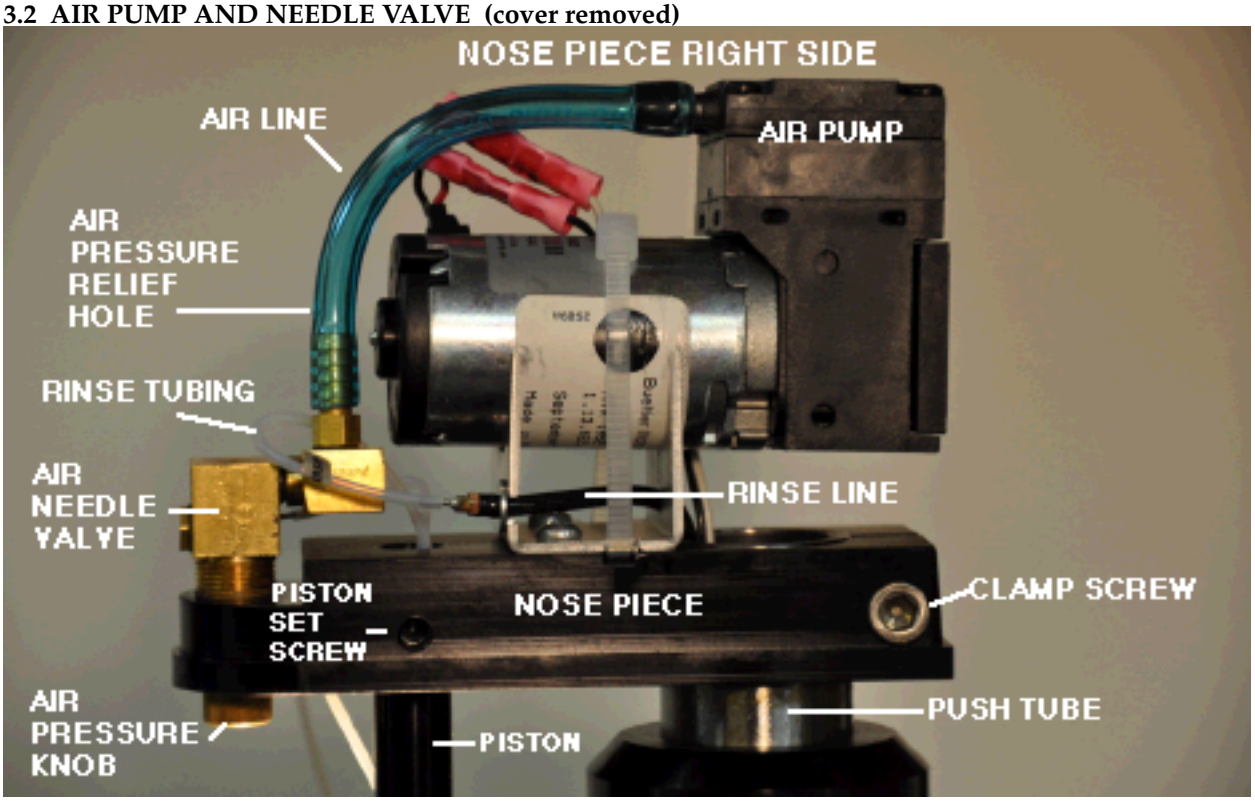
User Memories: 20. Each memory performs automatic fractionation of a specific gradient and contains up to 10 different segments with each segment containing user entered values of piston speed, distance per fraction, number of fractions and rinse.
Macro Record: The device will reproduce a manual run if it is recorded using this feature. The memory becomes one of the 20 memories listed above and can be edited and saved.

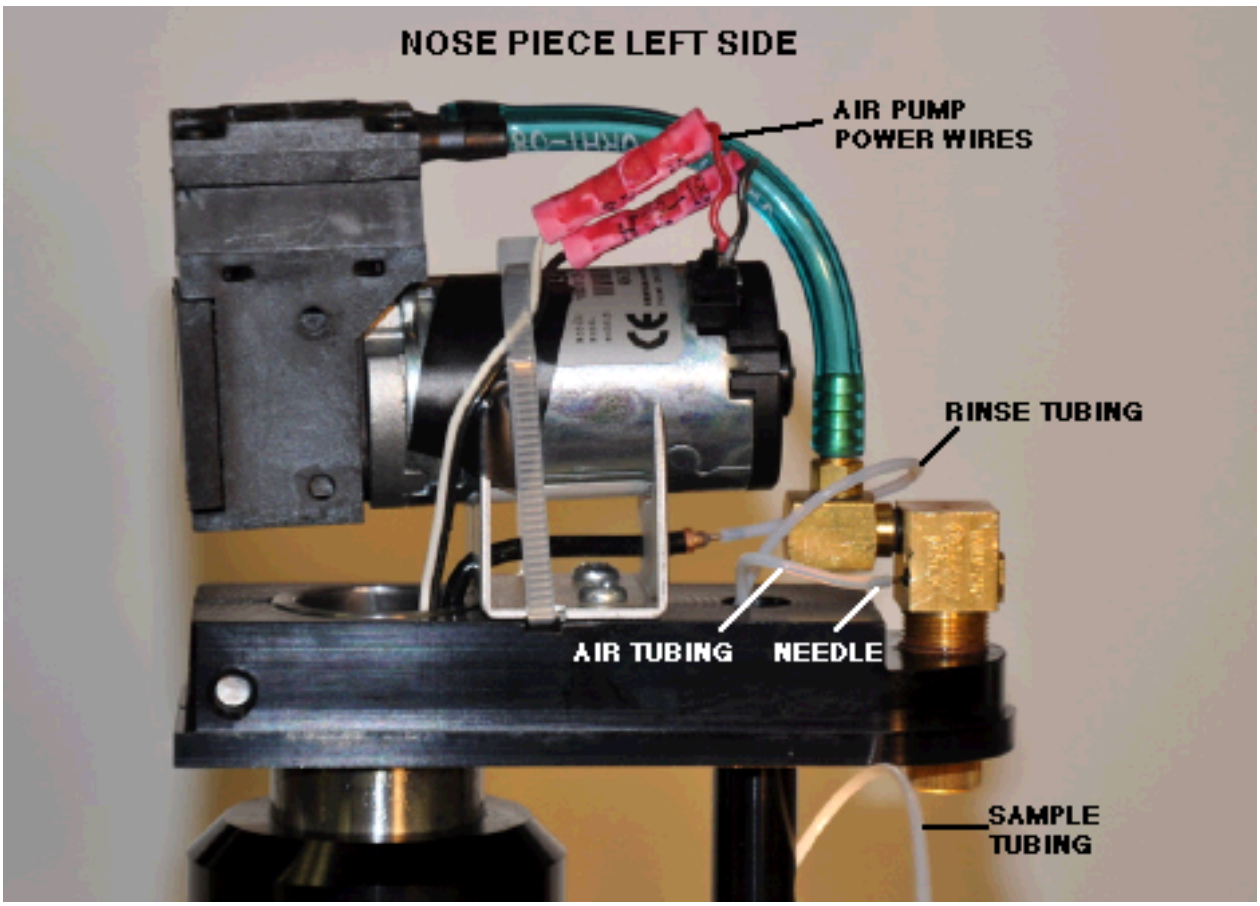
SECTION 3. PARTS DIAGRAMS

3.1 THE PISTON GRADIENT FRACTIONATOR

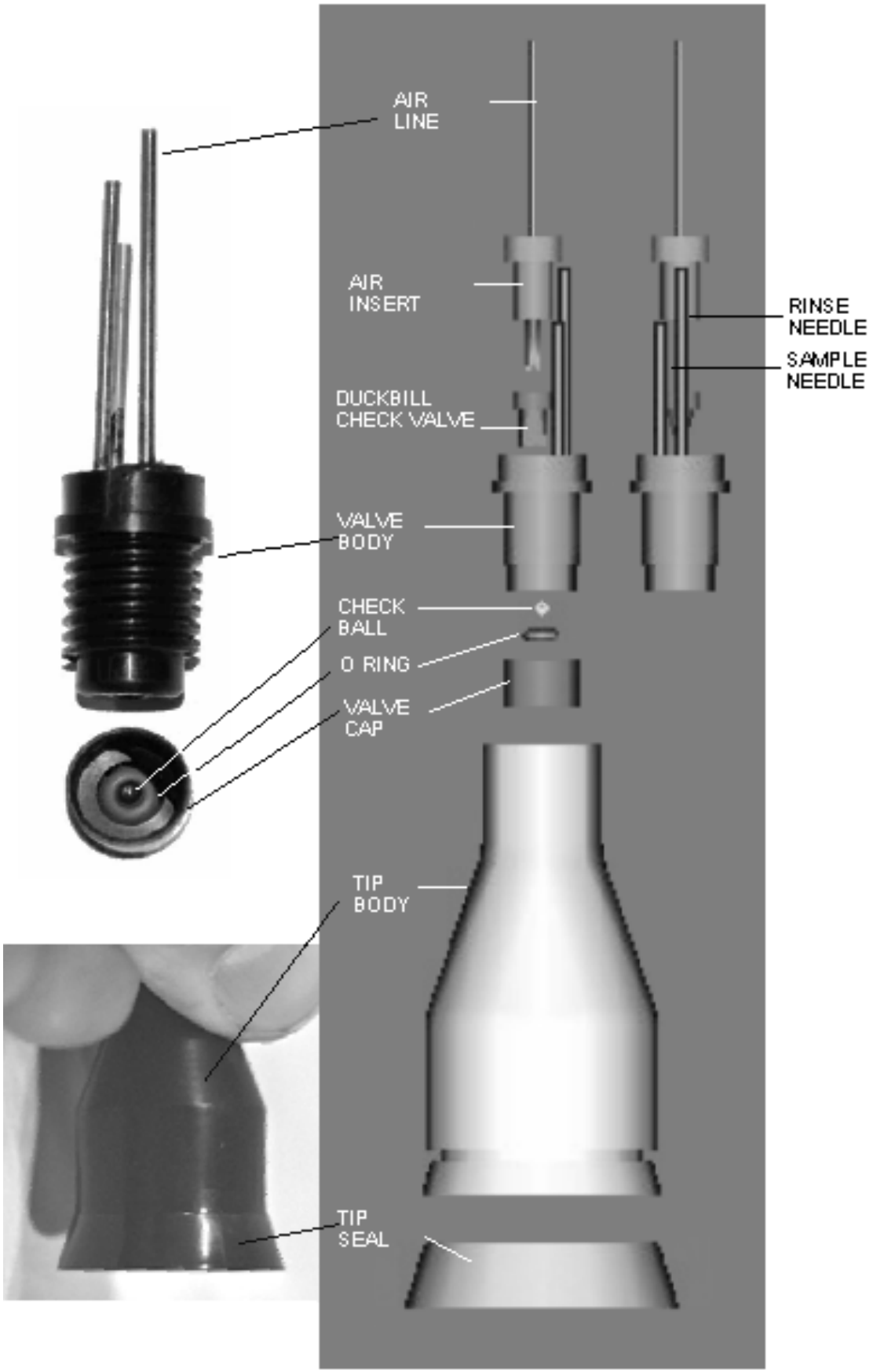


3.2 AIR PUMP AND NEEDLE VALVE (cover removed)

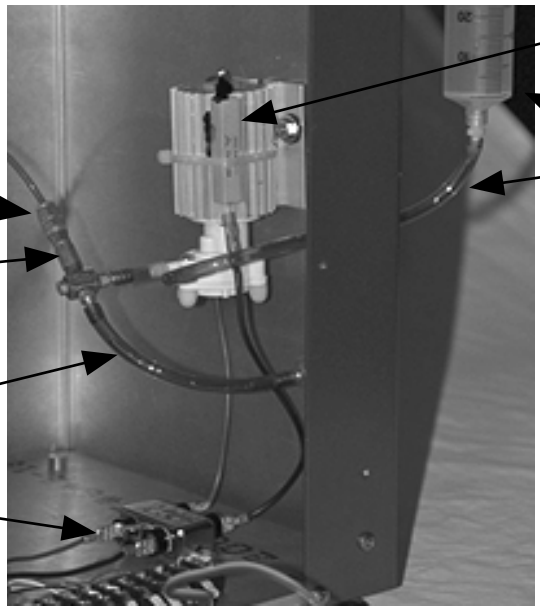




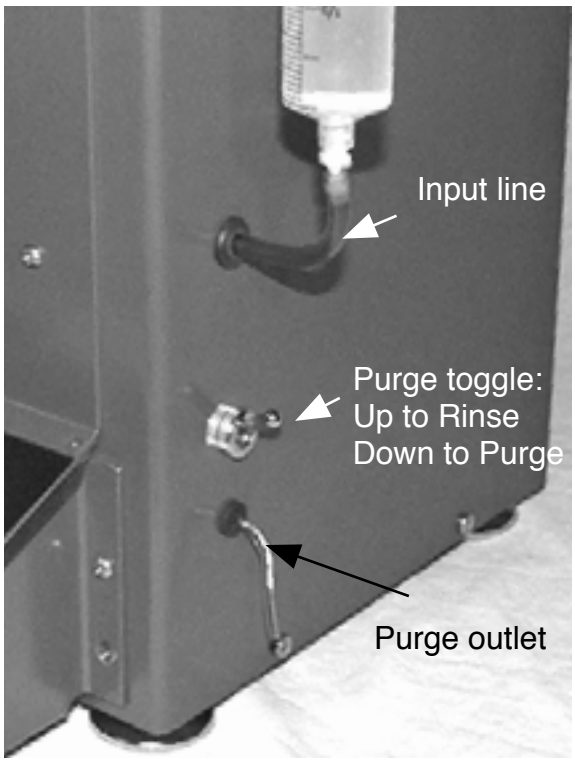
3.3 THE PISTON VALVE, TIP AND SEAL



3.4 THE RINSE PUMP AND PURGE SYSTEM



INSIDE VIEW
(Front panel removed)



OUTSIDE VIEW

SECTION 4. ORDERING INFORMATION

The Piston Gradient Fractionator™ gradient fractionation system.

4.1 Base Unit: Cat No. 152-001 90-120VAC input;
152-002 220-240VAC input
Includes the following accessories:

1 Manual
1 60 cc syringe rinse reservoir
4 feet
1 Set of 6 Allen Wrenches,
Spare check balls and 2 sizes of orings
1 Cursor for each tube holder
1 Spare light bulb
1 Spare valve assembly
1 SD card with manuals and backup software
1 m of spare Teflon tubing
1 fine marker
2 Working wings for extended working area
1 Front panel keypad/LCD display/computer
1 Fraction collector advance cable
1 Phillips screw driver
1 valve cleaning needle
2 sets of assembly screws; computer panel and wings
1 Water level syringe w/ stop
SD card w/ manuals, backup software and card reader
1 12VDC 6.6A transformer
1 power cord

4.2 Tube Holders: (each set includes 4 piston tips and disposable piston tip seals, tube holder, tube locking cap and cursor, box of 50 Seton centrifuge tubes)
151-125 25 mm dia. tubes for SW28 and like rotors.
151-116 16 mm dia. tubes for SW28.1 and like rotors
151-114A 14 mm tubes for SW41 and like rotors
151-114B 14 mm tubes for SW40 and like rotors
151-113 13 mm tubes for SW55 and like rotors.
151-111A 11 mm tubes for SW60 and like rotors (comes with a different narrow piston/valve assembly and spare)
151-111B 11 mm tubes for TLS-55 and like rotors

4.3 Tubes: (Seton PolyClear™ **required** for use with the fractionator)

151-525	25 x 89 mm tubes for SW28 and like rotors.
151-516	16 x 102 mm tubes for SW28.1 and like rotors
151-514A	14 x 89 mm tubes for SW41 and like rotors
151-514B	14 x 95 mm tubes for SW40 and like rotors
151-513	13 x 51 mm tubes for SW55 and like rotors
151-511A	11 x 60 mm tubes for SW60 and like rotors
151-511B	11 x 34 mm tubes for TLS-55 and like rotors

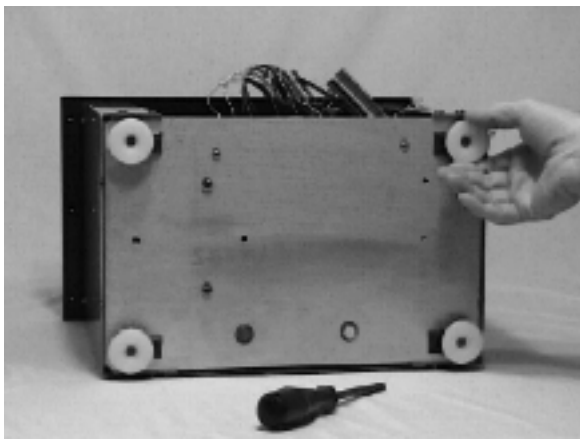
4.4 Spares:
151-2XX (XX is the mm diameter of the tube) Piston tip with seal
151-3XX-4 set of 4 seals for the xx diameter tubes
151-902 12V 20W Spot light with dichroic reflector
151-905 main piston valve assembly for air/rinse/sample
151-918 1.5 mm ø stainless balls and 002/003 silicone Orings for check valve assembly
151-925 1 m of replacement tubing for piston 1/16″ø Teflon
151-926 1 m of replacement tubing for rinse system 1/8″ø polyester

SECTION 5. INSTRUMENT SET-UP

5.1 INSPECT THE SHIPPING BOX. If there is any damage to the outside that corresponds to damage to the instrument, CALL BIOCOMP IMMEDIATELY.

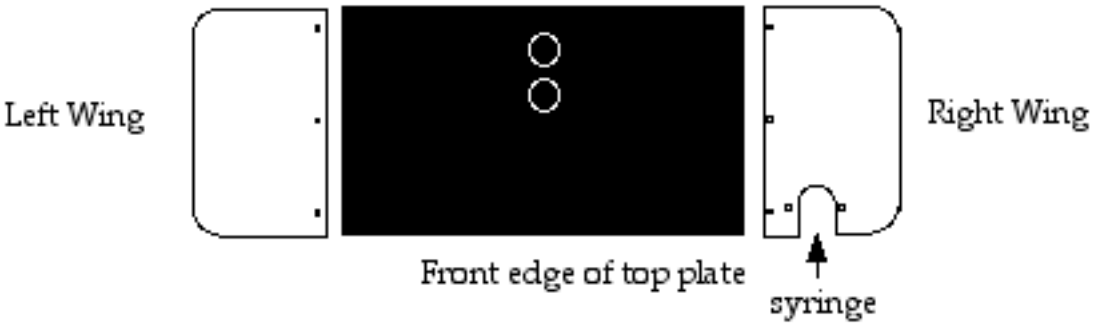
To unpack the instrument, first open the top of the box and remove all the small parts and loose foam. Then pull out the 4 pieces of foam from the sides of the carton. Pushing down on the box and pulling up on the sides of the top plate, remove the instrument from the carton. It is attached to a piece of plywood at the bottom that will pull out with the instrument. Use an adjustable wrench to remove the 4 bolts holding the plywood to the base of the fractionator.

Locate the 4 feet in the accessory pack and screw them into the holes left by the bolts. Level the instrument on the bench where it will be used by screwing the feet in or out as needed. Be sure that all the parts listed on the shipping papers are present.



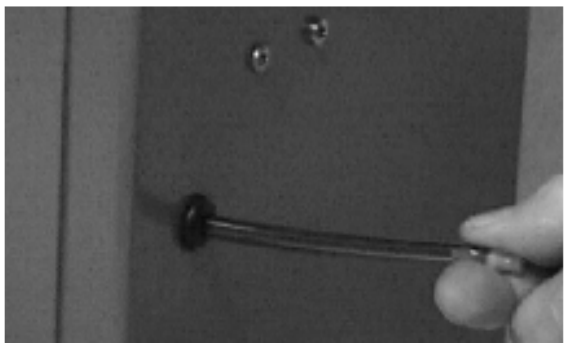
SAVE THE SHIPPING BOX FOR POSSIBLE RETURN OF THE INSTRUMENT.

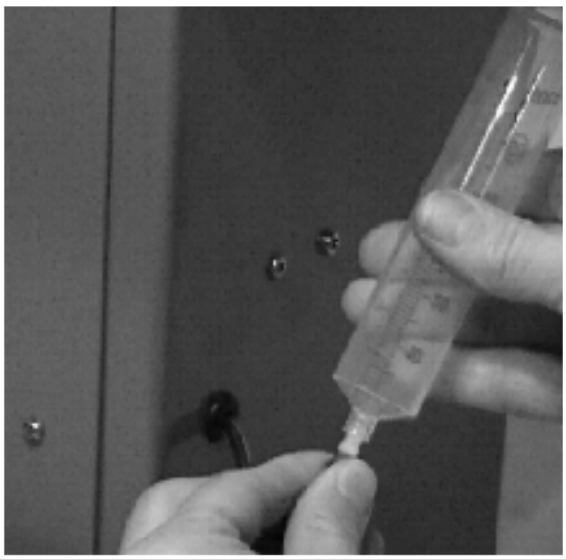
5.2 Assemble the Wings:
There are two black plates in the box. Both are 'D'-shaped and one has a large notch with screws on either side. The Notched plate is the right Wing and the other is the left Wing. Use the flat head screws and the 1/8" hex wrench to assemble them to the top plate. They extend the working area for tube racks.



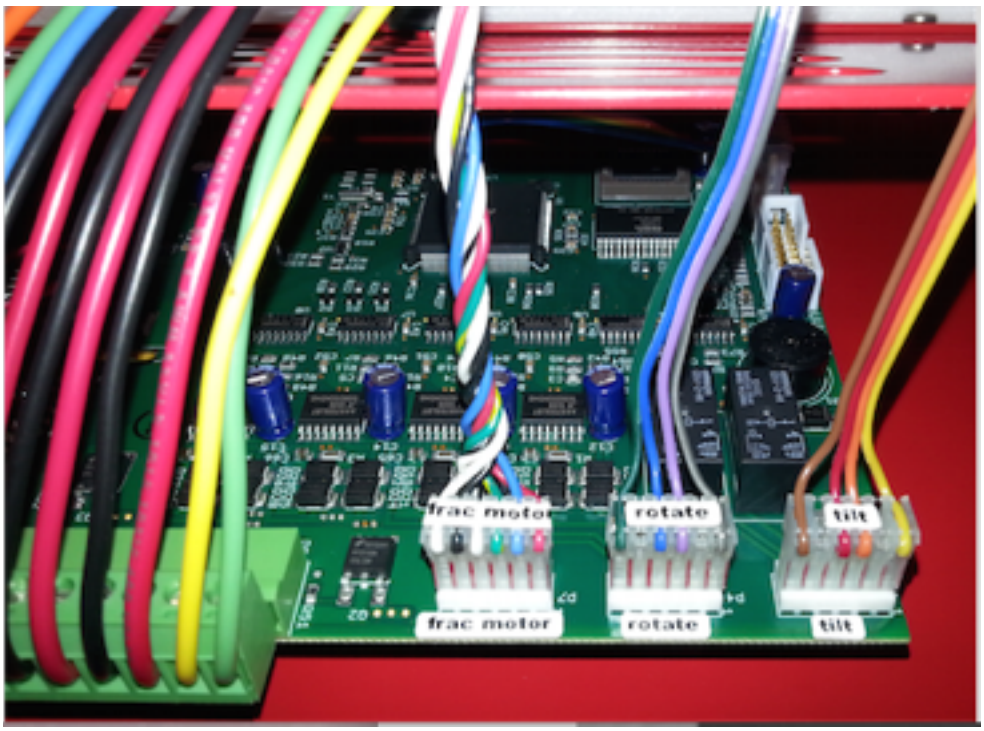
5.3 Attach the Reservoir:

There are two 60 cc syringes in the accessory kit. One is labeled **RESERVOIR** (no plunger) and the other **AIR**. Connect the Reservoir to the luer connector at the end of the blue tubing on the right side of the box (pull the tubing out first). Slide the flanges on the top of the syringe barrel under the screw heads on either side of the notch in the right wing. Fill the reservoir with **FILTERED** water, keeping a 1 l bottle close by. Never use the AIR syringe as a reservoir, since pulling the plunger out of the barrel creates small shreds of rubber that will clog the rinse system.





5.4 Assemble the computer panel. **First loosen the 4 front panel screws**. Find the green multi-wire plug and the smaller, white 6-wire motor plug(s) inside the main unit and expose them as shown below. The Station has FRAC MOTOR, ROTATE and TILT plugs. The PGF has all three sockets but only uses the FRAC MOTOR one. Turn the front panel upside down and insert the motor plugs onto their 6-pin sockets as shown

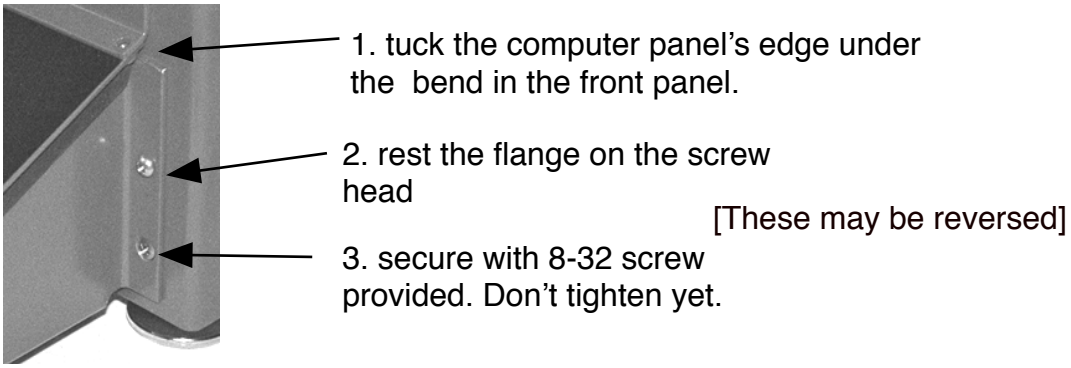


in the photo above. Note that the motor plugs have red stripes on one side. The red must face OUT, away from the white plastic back on the socket for the plug to fit. Be sure to align the plug directly over the socket so all the pins are covered.

Next, plug the green connector into the socket on the back edge of the circuit board. **Push it all the way in.** The plug can only be inserted with the scalloped edge up so there is no danger of inserting it incorrectly.

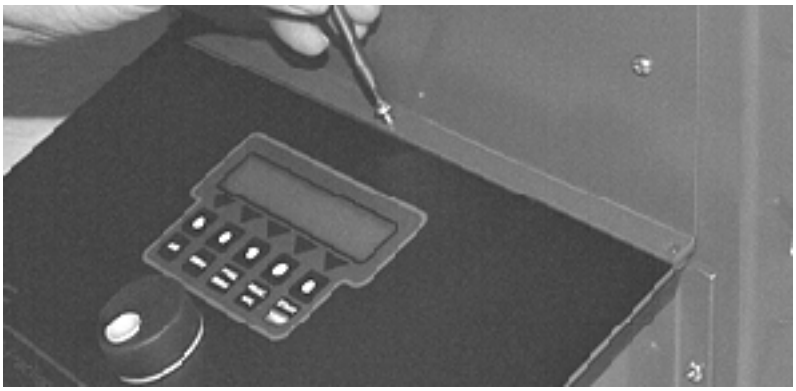
Flip the panel upright and find the Phillips screw driver and 2 of the 8-32 screws in the Accessory Pack.

Attach the front panel to the main unit by inserting its leading edge under the bent edge of the front panel and resting its two side flanges on the screw heads already installed on the side panels of the main unit as shown below.



Screw the two 8-32 screws into the flanges to stabilize the front panel on the unit.

5.5 Screw the computer to the bent edge of the front panel . Next screw the computer to the bent edge of the front panel using 3 more 8-32 screws as shown. When all 5 screws are started, tighten all 9 screws (4 top front panel, 3 top edge computer panel, 2 side computer panel).



5.6 Insert the power plug. Find the external power supply (12VDC Powergate transformer) in the box and insert its plug into the socket on the right rear corner of the instrument. We supply standard Euro or North American users with power cords that connect the transformer with the wall outlet. Other users must supply their own power cord. Be sure the switch above the plug on the back of the unit is off (0) and plug the power cord into the wall receptacle.

5.7 Turn the power switch on. After a brief delay, the display will light up a pale green and the display will read:

```
PGF FRACTIONATOR ver8.03
FRAC RINS STUP
```

This is the **MAIN MENU**

The row of 5 blank keys directly below the display are the "smart" keys whose values are determined by the words above; in this case, for example, the left-most key has the value "FRAC" for fractionate.

The dedicated keys in the second row have self-explanatory labels;
AIR turns on the air pump, forcing air into the collection tubing,
RINSE does the same for the water pump,
PROG. RINSE produces whatever series of rinse events is currently saved in the automatic rinse register,
FRAC. ADV. closes a contact to the fraction collector if connected by a cable and causes the collector to advance one fraction,
START/STOP key starts, stops or pauses any programmed activity, depending on the cycle the device is operating in. This is the "**PANIC BUTTON**".

SECTION 6. INSTRUMENT OPERATION

6.1. OVERVIEW: The instrument is preprogrammed and is now ready to run.

The instrument's functions will be described in two different, overlapping ways; one will be the **Menu Organization (6.2)** with a hierarchical view of the software flow and logic and the other will be the **Practical Manual (Section 7)**with step-by-step instructions.

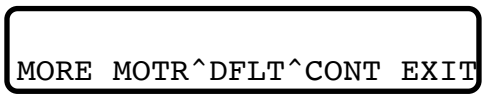
6.2 MENU ORGANIZATION

6.3 SETUP MENU

In this section, the functions of the various windows and keys will be described in a linear fashion - what happens when you press this key? An 'X' below a word in the bottom row of the display indicates that you should press this key. Begin by entering the SETUP menu. Press STUP:

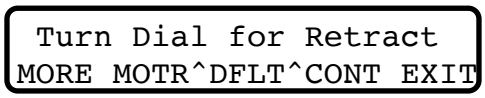
X

Center the dial and the display should now read. Press the MOTR key.



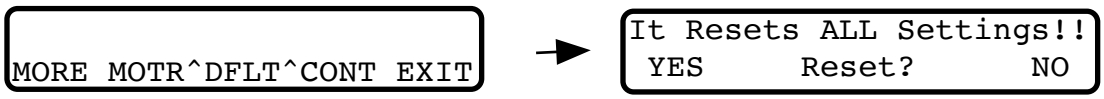
X

MOTR: This feature permits the rapid and complete withdrawal of the piston to the top of its stroke. To start the motor, spin the dial in any direction. When the piston is as far up as desired, press the **START/STOP** key or the **EXIT** key. The motor will stop on its own when it hits the upper limit switch inside the unit.



X

Press **DFLT**; This feature allows you to reset all the stored memories to their default values. This affects both **RINSES** and stored **MULTISTEP** runs, as well as **DIST** and **SPED** in the **SETUP** menu. Do not press this key unless you have recorded all your values, since they will all be lost. Press **EXIT** to return to the **SETUP** menu.



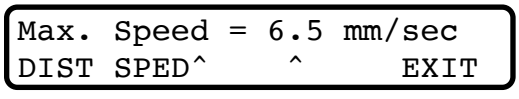
X X

CONT allows you to adjust the contrast on the LCD display. Press **EXIT** to return to the **SETUP menu**.

Press **MORE** to adjust the piston return stroke distance and the maximum UP and DOWN speed

X

DIST is the distance in mm that the piston will withdraw from the tube. When you begin a fractionation, you will press the **RESET** key to 0 the position of the piston tip just inside the tube. Setting the **DIST** to -25-30 mm will pull the piston out far enough to clear the tube holder so you can change tubes. Set the value by entering the desired value on the keypad. Press **EXIT** to return the **SETUP** menu.



X

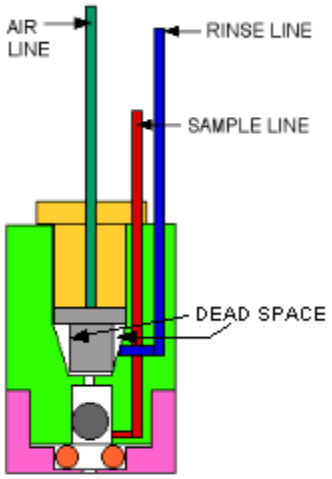
SPED adjusts the maximum speed the piston will travel in both the UP and DOWN directions. Press SPED, enter the numbers on the keypad and press EXIT. Set this value to 6.5 unless the piston stalls on the UP stroke. Lower speeds give more torque to help with lifting the piston.

6.4 RINSE MENU.

First a bit of theory. It has never before been possible to clean out the collection system between each fraction. Inside the Fractionator's valve assembly just above the piston tip is a set of check valves. A ball valve at the bottom of the valve rises off its oring seat as the piston moves down into the gradient, permitting the gradient to exit through the collection tubing (see Section 8 below).

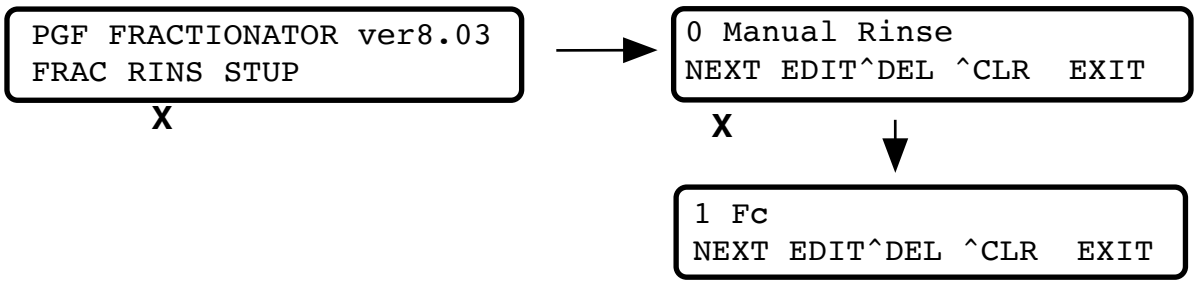
Just above the ball valve is an Air/Rinse injection chamber that permits the injection of air or rinse directly down onto the ball valve, driving it back down into its seat and sealing off the gradient. There is nowhere for the air or rinse to go but out the sample line. The injection chamber has a very small dead volume, but considering how small your fractions can now be, this dead volume may compromise resolution by hiding tiny amounts of the previous fraction. Water is not compressible, so if the injection check valve chamber is filled with water, no sample is allowed into the chamber, preventing contamination of this small volume.

Thus, if at all possible, your last command in the Rinse protocol should be **Wa** (see below). This short burst of rinse (~30 ul) will keep the first part of the **next** sample from entering the chamber.



VALVE ASSEMBLY

Now, on to the actual rinse protocols. Enter the **RINSE MENU** by pressing the **RINS** key on the Main Menu.



If you are scanning your gradients using a UV flow cell, the the rinse you MUST select is #1 Fc. If you wish to use the automatic rinse feature, press the **NEXT** key to scroll through some preprogrammed examples. There are 10 rinse registers in memory, with the first being manual (no programmed rinse), 6 registers containing a variety of preprogrammed rinses, and 3 empty registers for you to program. Within each programmed rinse, there are up to 10 possible events, each composed of a **function (A, W, D, F, P)** and a **duration (a**, **b**, **c**, **d**, **1-9** and **C).**

FUNCTIONS:

A = **AIR**; forces air through the collection system, removing sample and rinse.
W = **RINSE**; forces water through the collection system to remove residual sample.
D = **DELAY**; a programmed pause of a defined duration.
F = **FRACTION ADVANCE**; a circuit is closed for the length of time you specify to signal a fraction collector connected to the fraction advance cable supplied with the unit. Very short pulses **Fa** or **Fb** are usually long enough to get the collector to advance. **Fc** is the default pulse for DataQ event marking when recording UV profiles.
P = **PAUSE**; this is a true pause of indefinite duration. You must press the **START** key to continue. When you enter **P**, a '**C**' (for continuous) is automatically entered beside it.

DURATIONS:

C = Continuous
a = 50 msec = ~30 ul rinse*
b = 100 msec = ~125 ul rinse
c = 250 msec = ~225 ul rinse
d = 500 msec = ~305 ul rinse
1-9 = sec = ~.90 ml/sec rinse

* Rinse values will vary slightly, depending on the viscosity of the gradient solution ahead of the rinse in the collection tubing, pump characteristics, etc.. Calibrate your device by weighing a series of programmed rinses, for example 10 "a" rinses.

Pressing the NEXT key scrolls through 6 preprogrammed rinses which you may use as is or alter to your own needs. 0 MANUAL RINSE does nothing automatically. Push the air and rinse keys manually.

```
 1 Fc
NEXT EDIT DEL  CLR  EXIT
```

Fraction collector advance and event marker for GRADIENT PROFILER software when using the scan mode where the motor does not stop between fractions.

```
 2 A2DcFc
NEXT EDIT DEL  CLR  EXIT
```

Used for fraction collection where no rinse is desired. Air blows the residual sample out of the tubing, and after a brief delay, the fraction advances.

```
 3 D2A4PC
NEXT EDIT DEL  CLR  EXIT
```

Semi-automatic manual rinsing: delay to let the piston pressure to drop, air to drive the sample out, pause to let the user move to the next fraction.

```
 4 A2WcA2DcFc
NEXT EDIT DEL  CLR  EXIT
```

Fraction collection with a small rinse included in the sample: blow the sample out, follow with a brief rinse, blow the rinse out, advance fraction.

```
 5 A3D2W1A2WcA1WcA2WaPC
NEXT EDIT DEL  CLR  EXIT
```

Manual fraction collection with rinses separate from sample; blow sample out, pause to let user move tubing to rinse vessel, three short rinses, each driven out by air, a fourth tiny rinse to fill the duckbill chamber with water to prevent sample entry, pause to allow user to move to next sample (replace PC with D2 for continuous manual fractionation).

```
 6 A3FcW2A3Fc
NEXT EDIT DEL  CLR  EXIT
```

Used for fraction collection where rinse is collected separate from sample: blow sample out of tubing, fraction advance to next tube, rinse and blow, fraction advance to next tube, next sample, etc.

These 6 automatic rinse programs illustrate the flexibility you have in collecting your fractions. Which program you decide to use or develop yourself will depend largely on your specific needs. Read the following descriptions to find the protocol that most closely matches your method.

A. Scintillation Counting of Fractions: Most modern scintillation cocktails have a considerable capacity for water and solutes. Thus they permit extensive rinsing and the complete elimination of cross contamination between fractions. Use the Protocol#4 or #5, with Fc replacing PC if a fraction collector is used in #5.

B. Analysis of Proteins in Fractions: If you are doing gels of your fractions to determine their protein content, rinses are well tolerated, so use Protocol #5. Add 100% TCA to each fraction to a final conc. of 10% and put on ice for 30 min. Spin for 5 min in the microcentrifuge at top speed, gently suck off the supernatant and rinse the pellet with an equal volume of 10% TCA, spin again, remove the supernatant and rinse/spin twice with an 9:1 acetone:0.065M Tris (pH 7) mix and air dry. Resuspend in Laemmli sample buffer and run.

C. Enzyme Assays, Immunoprecipitations and other non-dilutables: If the dilution of fractions with rinses is a problem, you must either use **i)** no rinses, **ii)** add a small but consistent rinse to each fraction or **iii)** collect rinses separately from fractions and discard them.

i) No rinse. Protocol #2 blows sample out of the collection system, lets the pressure drop and advances the fraction collector. For manual collection, change **Fc** to **PC**, putting a pause between each fraction. You resume by pressing the **START** key. Alternatively, use **D4** so you don't have to press the START key; the next fraction will be taken after a 4 second delay.

ii) Small Rinse; If the addition of a defined, small amount of rinse is tolerable, use protocol #4, changing the duration of **W** to suit your needs.

iii) Discard the rinses: Protocol #6 drives the sample out with air, advances the fraction collector, rinses into a new tube and advances the fraction collector for the next fraction.

D. UV scans -Continuous Fractionation. When obtaining UV scans of polyribosomes, for example, two things are needed: advancing the fraction collector and marking the saved profile in the Gradient Profiler software with each fraction advance. Fractionator software versions 8.03 and beyond and Gradient Profiler versions 1.74 and beyond have taken control of the fraction collector (event marks) away from the Fractionator/Station and given them to the Gradient Profiler software. See section 6.12 below for full details.

In any case, the RINS must be set to #1 Fc, as this is the only RINS that does not stop the piston at the selected DIST/fraction interval, allowing continuous fractionation and a steady profile without breaks.

*This is a special mode that automatically engages when **Fc** is the **only** rinse*

It differs from all other modes because the piston motor never stops at the end of each fraction. The only thing to happen is a pulse (**Fc**) sent to the fraction collector cable at the defined distance. This feature is used in either the SNGL or MULTI automatic modes (section 6.14). Remember that the RINS protocol showing when you exit the RINS menu is the rinse used in SNGL, so leave R#1 (**Fc**) showing. For MULT runs, use R1 (**Fc**) as the only rinse in the protocol.

6.5 EDITING RINSE PROTOCOLS:

Scroll through the 9 adjustable memories (Protocols 1-9) with the **NEXT** key to the one you want to change. There are three ways to edit your rinses;

- You can press the **EDIT** key to obtain the blinking cursor and then using the knob to move the cursor to the event or time you wish to change, press **EDIT**, dial in the change you want, press **ENTR** again to fix it in memory. All entered changes are recorded in memory, erasing old values.
- To remove an event from the series, position the cursor over the event or the time and press the **DEL** key. Any events to the right of the deleted one will fall back one register.
- If you want to delete all events in this rinse register, press the **CLR** key.

Don't forget to press the **ENTR** *key after entering the time value for your last event. Failure to do so will result in the loss of that event from the memory when you* **EXIT** *this menu.*

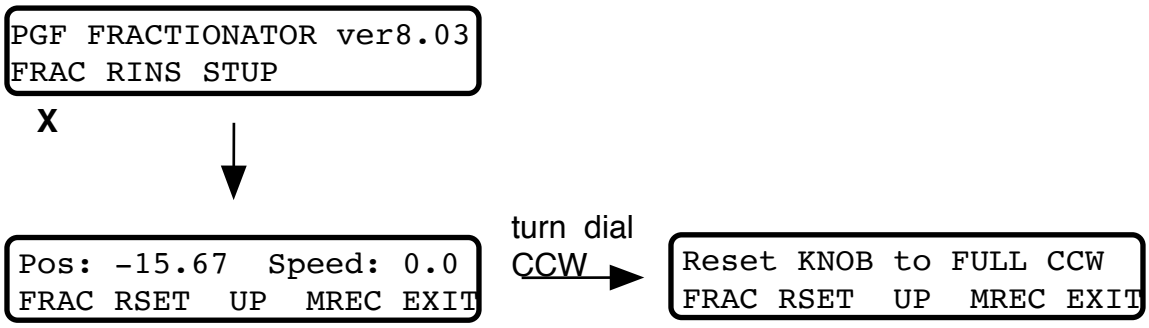
*The register you leave showing on the display when you press the **EXIT** key to return to the **MAIN MENU** will be the default programmed rinse for the fractionation that follows.*

This completes the **RINSE** section. This feature is unique among fractionators and its proper use will greatly enhance the purity and reproducibility of your fractions. Press **EXIT** to return to the **MAIN MENU**.

6.6 FRACTIONATION MENU

You are now ready to begin fractionation. Use the **MOTR** function in the **SETUP** menu described above to lift the piston up out of its shipping position. Once the piston is high enough to clear the top of the tube holder, press **STOP** and **EXIT** to return to the **MAIN MENU**.

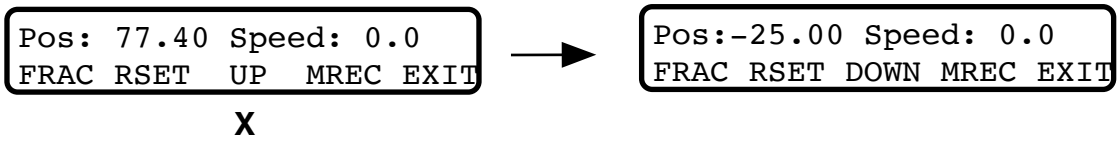
Press **FRAC**; the dial is now a motor velocity control. To start the motor, turn the dial fully Counter clockwise (CCW). If you now turn it clockwise (CW), the piston will begin to move downward, with velocity increasing the further you turn it. Counter clockwise rotation slows it down and stops it.



The motor velocity (Speed: 0.00 mm/sec) increases to 6.5 mm/sec as the dial is turned 270° CW. Take a few minutes to get the feel of this control. At the top and bottom of the piston stroke are two limit switches that prevent piston movement beyond its desired range.

There are two ways to raise the piston: A. **Before** you reach the lower limit switch, and B. **After** you reach the lower limit switch.

A. Raising the piston before you reach the limit switch: To raise the piston, turn the knob to full CCW and press the **UP** key:



Upward motion is automatic in this mode. The piston will rise until one of three things stops it:
1. It counts down to 00.00 on the display (the last reset point) and then continues a further (-)XX mm before stopping, where -XX is the **DIST** (default = 30.00 mm) you set in the **Setup Menu**. If you want the piston to go up further, press **UP** again, the piston will go up until the upper limit switch is triggered or you press the **STOP** key.
2. It hits the upper limit switch.
3. You press the **STOP** key. This returns the instrument to its **DOWN** state; if you rotate the dial clockwise, the piston will move downward.

B. After you hit the lower limit switch, the motor reverses automatically and the piston withdraws from the tube until it reaches (-) DIST or hits the upper limit switch. If you need further upward movement, press the **UP** key again.

6.7 MANUAL PISTON CONTROL

There are three uses for **MANUAL** fractionation:

- To position the piston at the start of a new gradient
- To manually fractionate the gradient
- To manually fractionate a gradient while recording it into memory using the **MREC** function.

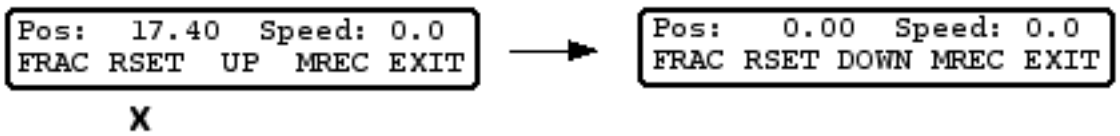
6.8 POSITIONING THE PISTON TO START A RUN: THE FIRST DROP

The piston's position shown on the display is relative to a 0 point you set with the **RSET** key. Ideally, this point is the top of the gradient. In practice, however, it is difficult to see the top of the tube because it is hidden by the holder cap or because the meniscus reflects light very strongly. There are two ways to set the top of the gradient (the 0 point on the display), and both are OK to use.

6.8.1 The first is called the **FIRST DROP**. It is the position of the piston that brings the **FIRST DROP** of the gradient to the end of the collection tubing. You will immediately recognize that the piston will be some distance down into the tube before the first drop appears. The trumpet tip and the tubing must fill before the drop shows up.

It will take some practice to become proficient at catching the first drop. The idea is to move the piston rapidly down through the cap into the tube and to slow down to a crawl as the piston enters the gradient itself. Use the dial to obtain the desired speed. Once the tip has entered the gradient, watch the tubing for the first signs of liquid moving through it. When the first drop appears, spin the dial counter clockwise or press the **STOP** key. The larger the centrifuge tube diameter, the faster the liquid will move through the tubing at a given piston speed due to the larger volume being displaced.

Once the first drop appears at the end of the sample tubing, press the **RSET** key to 0 the position indicator:



X

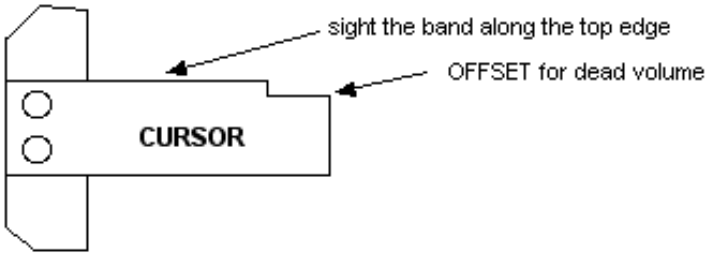
This is the top of the gradient. Now you can see the need for the **DIST** function in the **SETUP** menu. To be able to remove the tube holder after the run is finished, the piston must rise some distance above this 0 point.

6.8.2 The second method is called **GOING TO THE LINE**. It is identical to the FIRST DROP, except that you draw a line on the tubing near the piston and stop the piston when the meniscus appears at the line rather than at the end of the tubing as a drop. This is the method used in UV profile scanning to limit the amount of gradient lost before scanning starts. If you mark the position of the meniscus on the tape using the cursor (see section 6.9 below), you can quickly descend at high speed, slowing down as the bottom edge of the seal approaches your meniscus line and stopping when it hits the line. The top of the gradient now lies at the top of the piston tip, at the bottom end of the valve. Proceed down slowly from this point and you will have a better chance of seeing the liquid meniscus moving toward the line on your tubing. Hints: Before you begin fractionation, open the air valve in front of the piston, give the air a long hard blast to dry the tubing, then close the air valve and proceed to lower the piston. Try this with a tube filled with water until you get used to it.

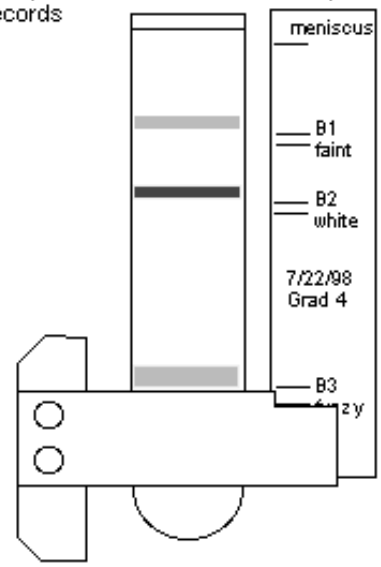
UV-Profiles: skip to section 6.11&12 below

6.9 MANUAL FRACTIONATION.

If you can see the bands of interest by scattered light or some other means and want to recover them intact, then this simple form of fractionation is the one to use. Place a piece of tape (Time tape) on the tube holder to the right of the window. Find the cursor for this size tube in the Accessory Pack and use it to mark the position of the meniscus and the top and bottom of each band. The tape will be your permanent record of the gradient and is simply transferred to your experimental notes.

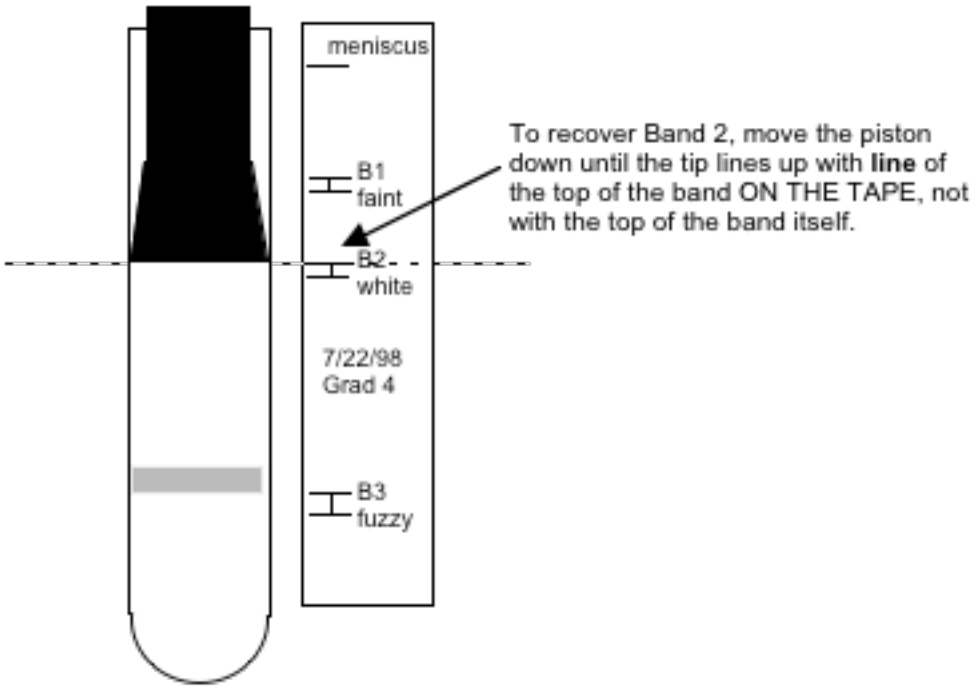


Mark the bands on the tape with the marker provided;
Record the top and bottom and the intensity of the band
for your records



It is a good idea to number the bands from top to bottom and describe their intensity and sharpness using the marker provided and a code you invent. An example is shown above.

To recover a band of interest (you can go directly to the band regardless of its location in the tube), use the dial to move the piston down until the **leading edge of the piston tip lines up with the line marking the top of the band**.



Because of the offset in the cursor, the top of the actual band will be up inside the trumpet tip at the point of **GRADIENT CAPTURE at the bottom of the valve**. Move slowly as you approach the top of the band so as to not disturb it. Rinse manually by pressing the **RINSE** and **AIR** keys a few times and leave the

tubing empty with a long blast of air. Alternatively, press **PROG. RINSE** to activate the automatic rinse, the one you left active when you exited the RINS menu (the rinse must end with A2-A4 to leave the tubing dry)

Now place the end of the tubing in the receptacle for the band (an eppie) and move the piston down slowly until the edge of the tip is at the **BOTTOM MARK ON THE TAPE**, then blow out any sample left in the collection tubing.

Recover all the bands you desire using the same strategy; move the piston rapidly (~1 mm/sec) when there is some distance to go, slow down (.3 mm/sec) as you approach the top of the band, stop, rinse, blow dry, move slowly (.2 mm/sec) through the band, stop and blow out the band in the tubing.

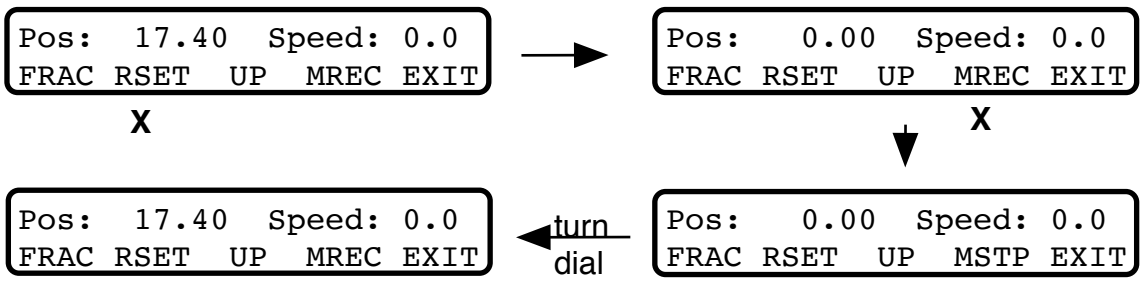
If you reach the lower limit switch at the bottom of the tube, the piston will automatically stop and withdraw to **-DIST.**

The position showing is the total distance in mm that the piston has moved since the RESET key was pressed. Press **UP** and the piston will retract until it reaches the -XX mm distance above the last RESET = 0.00 position you set.

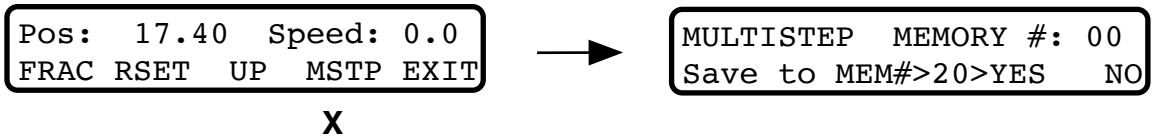
6.10 MACRO RECORD.

This feature combines manual fractionation with memory. The fractionator will watch you manually fractionate a gradient and record your moves in a designated memory register. It knows when you take bands because it looks for rinse, air, programmed rinses, and frac advances. It automatically sets the motor speed to correspond to the distance between starts and stops. To repeat the same run on another tube, you simply recall the memory register you recorded it into, edit it if you like, and rerun it.

In the Manual Fractionation Mode; obtain your first drop, press **RSET** to 0 the distance and then press **MREC.** If you fail to press **RSET**, a prompt alerts you to do so**;**



Perform Manual Fractionation as described above, with manual or automatic rinses to mark the end of one segment and the beginning of the next. Nine gradient segments can be recorded into memory, so up to 5 bands separated by 4 discarded layers, or up to 9 consecutive bands, can be recovered.



After you have performed your last rinse, press **MSTP**;

Enter the memory register (00-19) using the keyboard and press **YES**. Pressing **NO** will return you to the Manual Fractionation window without saving the run. If you pressed **SAVE**, this run is now entered as a **MULTISTEP RUN** (see below) and can be recovered and run automatically.

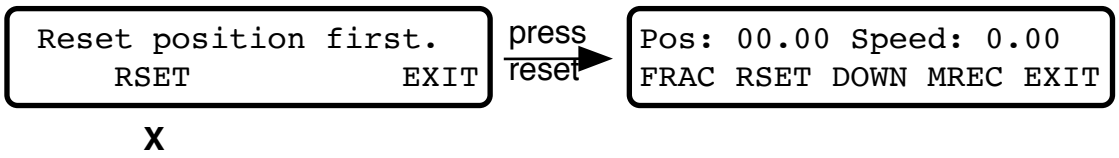
6.11 AUTOMATIC FRACTIONATION.

There are two types of automatic fractionation;

• **Single Step:** the gradient is fractionated from the top down into "**n**" identical fractions separated by the same automatic rinse (or manual rinse if desired) you left active when you exited the RINS menu. The run is **not** entered in memory.

• **Multistep:** 1-10 segments like the single step segments above (consisting of a piston speed, a distance per fraction and a number of fractions) are recorded in memory in advance of fractionation and run automatically.

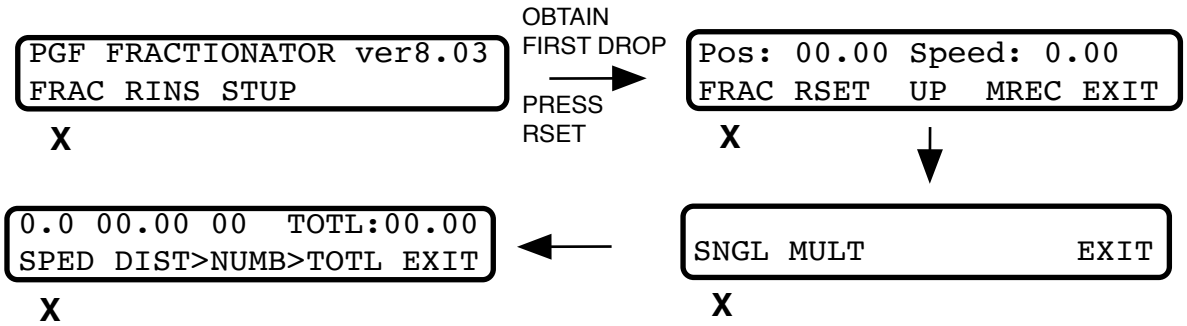
In either type, if there is a X.XX position on the display and you press FRAC for the second time, you will be prompted to press reset:



6.12 SINGLE STEP RUNS.

In this mode, the entire gradient will be fractionated into "n" equal fractions with the active RINS between each fraction. If the RINS is #1(Fc), the piston will not stop between fractions.

Obtain the first drop or "go to the line" and press **RSET** to 0.00 the position on the display. Press **FRAC**, **SNGL** to reach the single step programming window:



Press **SPED**, enter the desired value using the keypad (0 - 2.0 mm/sec), press **SPED** again to save the value.

You have two choices for setting the number of fractions and the distance of each fraction:

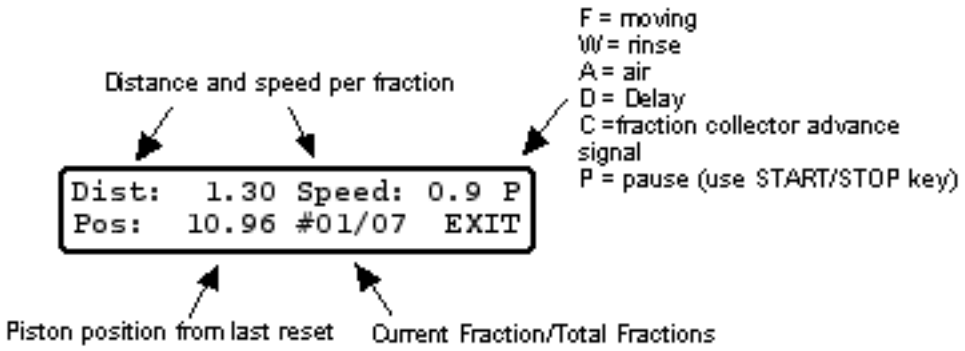
1. Press **TOTL**, enter the total distance in mm you want the piston to travel from the bottom edge of the seal to the bottom of the stroke (add 2 mm to the distance to the bottom of the window if you want the piston to harvest the maximum amount of liquid from the tube). Press **TOTL** again. Now press either **NUM** to enter the number of fractions desired or **DIST** to enter the distance of each fraction. The remaining value will be automatically entered. If you press **TOTL** again, both **NUM** and **DIST** are reset to 0.

NOTE: Gradient Profiler software v1.74 and greater require that the Total distance+10 mm = TOTL and that NUM = 1 so that the entire gradient will be harvested in one large fraction with the fraction collector advance pulses being controlled by the GP software itself.

2. Press-enter-press both **DIST** and **NUM** and the **TOTL** distance will be automatically calculated.

When you are satisfied with the numbers, press **START**.

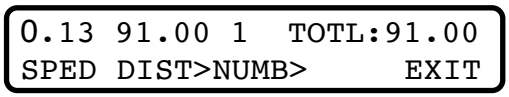
The display now shows an active window that allows you to follow the run.



Dist: 1.30 Speed: 0.13

6.13 UV PROFILES:

Remember to leave RINS #1 (Fc) as the only rinse function showing when you exit the RINS menu, as described above. This will prevent the motor from stopping during the profiling.



A typical SW41 SNGL run is shown here: .

The only fraction advance pulse sent to the fraction collector and the Gradient Profiler software is the START pulse. The Gradient Profiler software sends the remaining pulses.

If the total distance exceeds the distance to the end of the cylindrical part of the tube, the piston will hit the lower limit switch inside and automatically stop and withdraw.

When the run is finished, and the piston has not withdrawn, press **EXIT, EXIT, EXIT, EXIT, UP** and the piston will retract.

6.14 MULTISTEP FRACTIONATION.

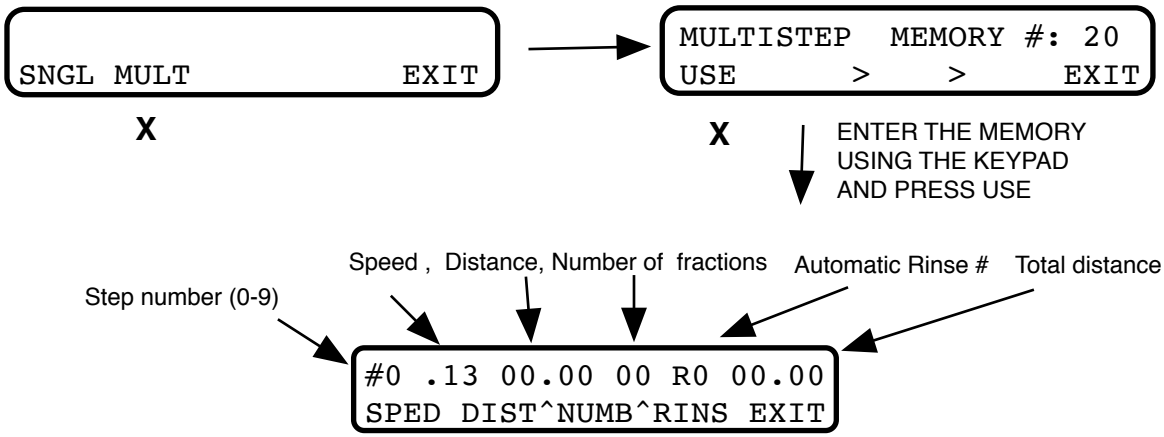
The goal here is to **pre-enter** a gradient containing 1-10 segments into memory. Each segment resembles a single step run having its own speed, distance, number of fractions and rinse. This feature permits fine fractionation of important areas of the gradient, while taking fewer fractions in areas that are less interesting. This reduces sample processing while preserving the absolute distance between the first drop start and any given fraction.

Two hints will be helpful; At the start of fractionation, the **tip** and **tubing** will be contaminated by the debris at the top of the gradient, so it is **important to clean them out between gradients**.

In your first section, take a few fractions (4-6) in the first 10 mm below the meniscus and use a rinse protocol that has several rinses, like #5 for example. This will eliminate contamination from the concentrated sample material on top of the gradient into subsequent fractions.

The second tip is to obtain at least 10 fractions for each peak (a rule from HPLC), so you can observe subtle changes in its shape and height. The sharpest bands we have fractionated with phage T4 heads are about 1 mm thick and require 0.3 mm fraction sizes. We have resolved two peaks separated by 1.5 mm. Broader bands can tolerate larger size fractions.

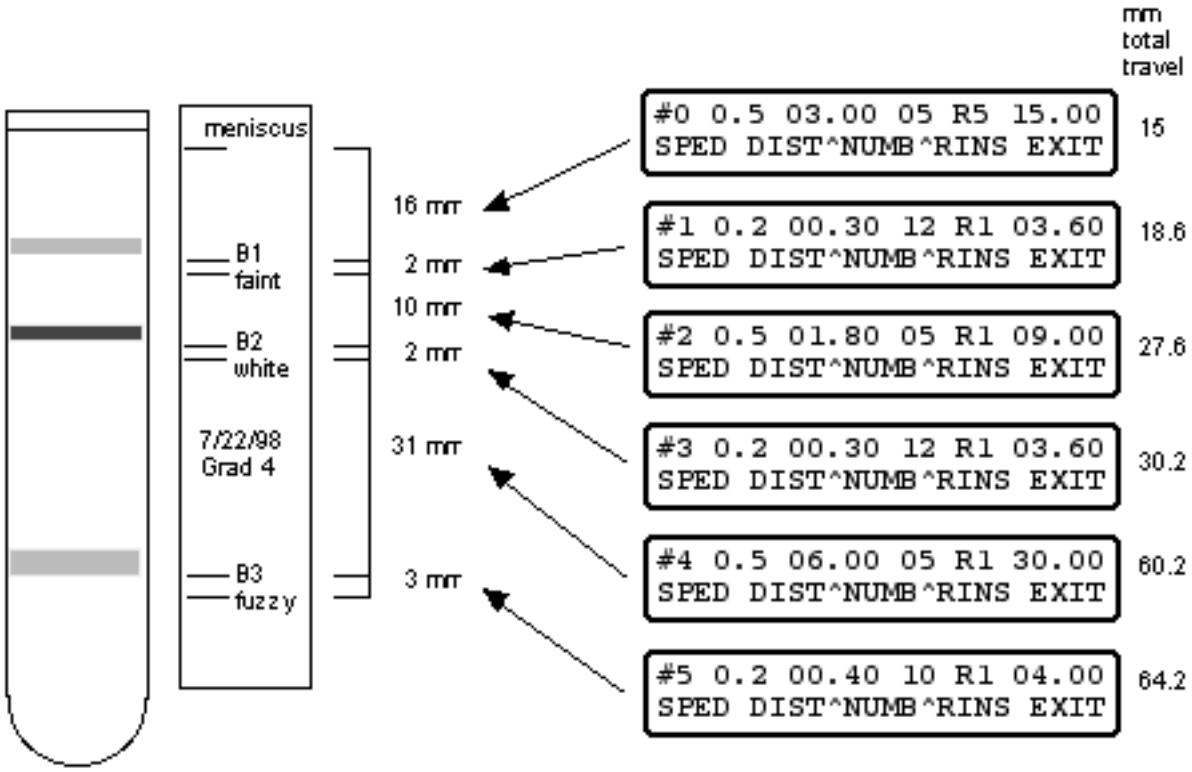
To enter the gradient into memory in advance of the run, from the **MAIN MENU**, press **FRAC,** obtain the first drop or "go to the line" by manually moving the piston down with the dial, press **RSET** to 0 the display, and **FRAC, MULT** to reach the **MultiFraction Programming Window**. Use the keypad to enter the memory register (1-20);



As before, pressing the **SPED, DIST or NUMB** keys and entering the value using the keypad allows you to set the piston speed, etc. for this segment. The 4 digits in the right corner are the total distance of **this** segment, not the combined distance of all segments.

The next segment is entered by turning the MAIN dial CW (be sure that none of the SPED, DIST or NUMB keys are blinking). The dial can be used to scroll forwards or backwards between segments. #0 is the first and #9 is the last segment.

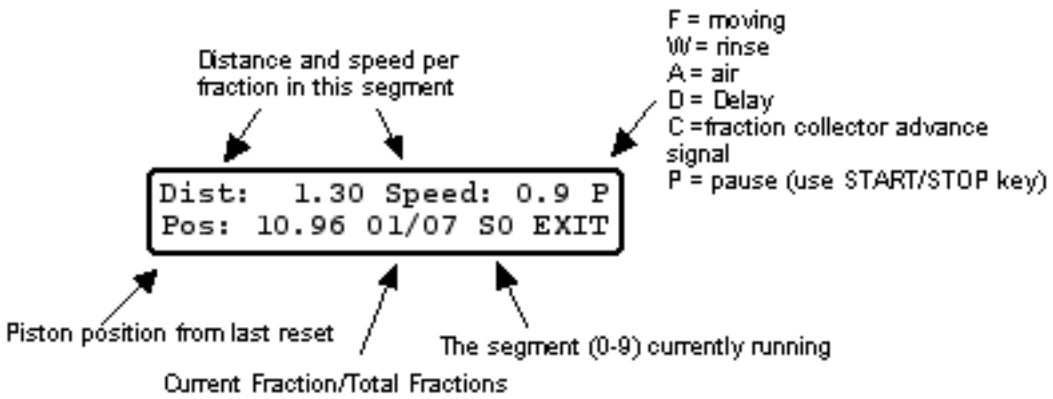
Let's revisit the manually fractionated gradient in the previous section. Rather than simply recover the bands intact, we want to analyze the whole gradient with emphasis on the 3 band areas. Measure the distances on the tape to determine how to set the run up:



With 49 fractions, this run would be at the upper limit of what you would do routinely. However, it illustrates the principle of taking many samples in areas of interest and fewer samples in between. Anticipate the arrival of a band before you can actually see it by extending refined fractionation slightly above and below the visible band.

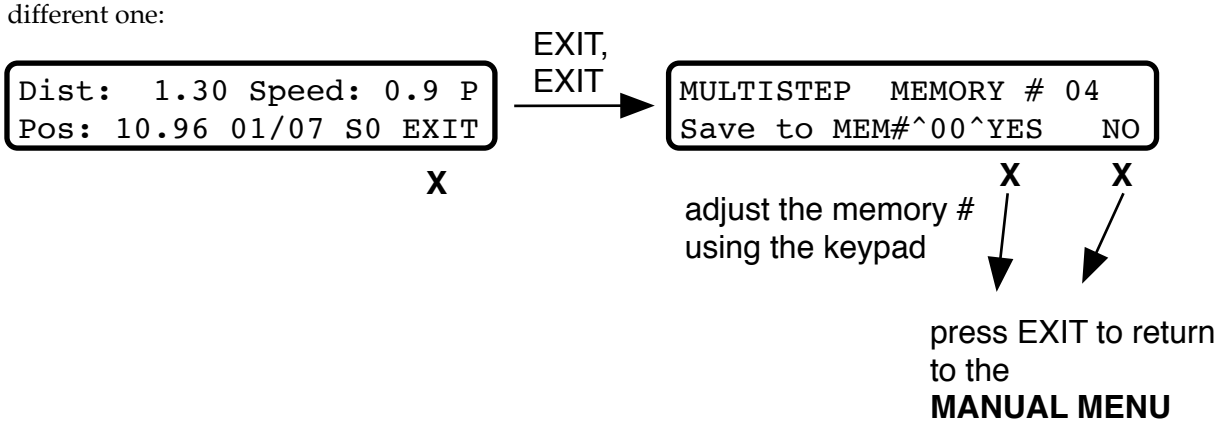
If your bands are not visible, fractionate one gradient evenly and fairly crudely to see where the particle(s) of interest lie and then refine your fractions in subsequent runs.

If you want to run a gradient already recorded in memory without changing it, in the MULT menu, enter the memory register (1-20) and press **START**. Otherwise, scroll through the various steps and adjust the values and then press **START**. Fractionation will proceed, with the preprogrammed rinses separating each fraction. Have your tubing and vials ready. Once the run has started, the display will look like this:



To abort a run in progress, press the **STOP** then **EXIT** key.

At the end of complete run, press **EXIT, EXIT**. If you have changed any of the run parameters before the run, you will be asked whether you want to save the changes in the present memory register or a different one:



6.15 MANAGING THE DATA

Graphing results obtained with this type of uneven fractionation requires some care because the different samples do not represent equal volumes and are not equally spaced as they probably have been in your past work. A spreadsheet is invaluable here because it allows you to compensate for both differences before plotting a graph.

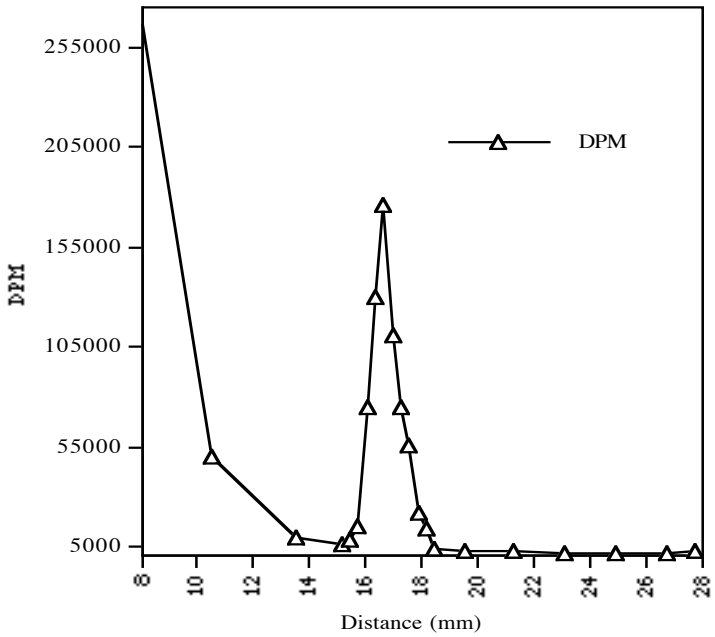
There are two possible types of data:

- the same aliquot is removed from every fraction you took and analyzed (constant volume).
- The entire fraction is analyzed; (whole fraction).

A. Constant volume: When you withdraw a fixed volume from each sample for your assay, you only need be concerned about where to plot the value on a graph. Place each data point in the center of the fraction it originated from.

frac no.	counts	frac size (mm)	center point (mm)	center point (mm)
1	5329847	3	0.5*C2	1.5
2	897943	3	D2+0.5*C2+0.5*C3	4.5
3	309801	3	D2+0.5*C3+0.5*C4	7.5
4	49661	3	D2+0.5*C4+0.5*C5	10.5
5	9801	3	D2+0.5*C5+0.5*C6	13.5
6	6239	0.3	D2+0.5*C6+0.5*C7	15.15
7	7941	0.3	D2+0.5*C7+0.5*C8	15.45
8	15539	0.3	"	15.75
9	75142	0.3	"	16.05
10	129682	0.3		16.35
11	176321	0.3		16.65
12	110863	0.3		16.95
13	74890	0.3		17.25
14	55420	0.3		17.55
15	21897	0.3		17.85

The formula "D2+0.5*C2+0.5*C3" takes the center point of the previous fraction (D2), adds the distance to the end of that fraction (.5*C2), and then adds the distance to the center of this fraction (.5*C3).
This produces the graph below showing the concentration of data points around the peak area:



B. Whole fraction: If the whole fraction is assayed *and* you change the volume of your samples during fractionation, you need to compensate for both volume and position. The problem is solved by expressing the data *per unit volume* by dividing the counts by the distance of that fraction. The position of the data point is calculated as before:

frac no.	counts	frac size (mm)	dpm/mm	center point
1	1258692	3	"=B2/C2"	1.5
2	897943	3	299314	4.5
3	309801	3	103267	7.5
4	49661	3	16554	10.5
5	9801	3	3267	13.5
6	6239	0.3	"=B7/C7"	15.15
7	7941	0.3	26470	15.45
8	15539	0.3	51797	15.75
9	75142	0.3	250473	16.05
10	129682	0.3	432273	16.35
11	176321	0.3	587737	16.65
12	110863	0.3	369543	16.95
13	74890	0.3	249633	17.25
14	55420	0.3	184733	17.55
15	21897	0.3	72990	17.85
16	14360	0.3	47867	18.15
17	4292	0.3	14307	18.45
18	2897	1.8	"=B19/C19"	19.5
19	2471	1.8	1373	21.3
20	1755	1.8	975	23.1
21	1293	1.8	718	24.9

SECTION 7. PRACTICAL MANUAL

This section is intended to be a hands-on manual and assumes that you have familiarized yourself with the software, since many operational details have already been covered in the Menu section. It begins with a list of how-to's and ends with a step-by step procedure for fractionating a gradient.

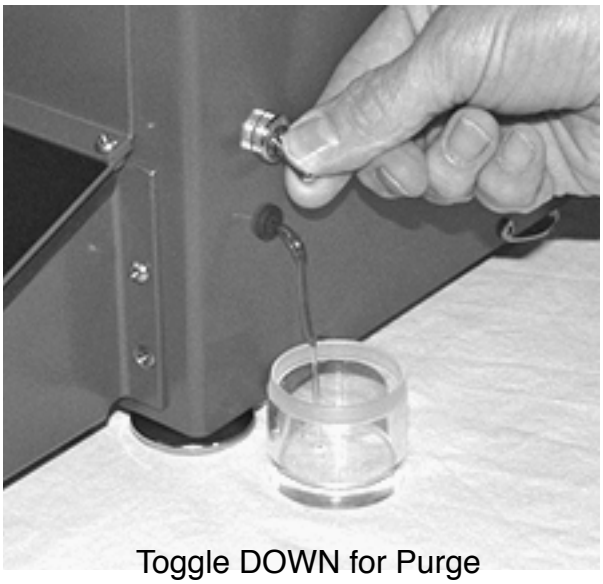
7.1 Changing the piston tip:

The unit as shipped has the valve assembly installed in the piston, but lacks a piston tip. These are found in the accessory kit and simply screw on to the end of the piston. **Do not over-tighten**, finger tight is fine. After each gradient, remove the tip, and examine it for any liquid in the threaded cavity. If you find liquid here, it means that the oring seal between the tip and the bottom of the valve assembly has failed. Check for dirt on both parts and substitute a new tip if the leak persists. Clean the tip before reinstalling it.

7.2 Priming the water pump:

Just inside the instrument on the right side is a water pump that forces rinse from the syringe reservoir into the piston and out the collection tubing. The pump must be primed before it can generate any flow, so we have installed a bypass or purge valve that shunts the pump to the small black tubing on the right side of the box.

Toggle UP for Tubing Rinse



Toggle DOWN for Purge

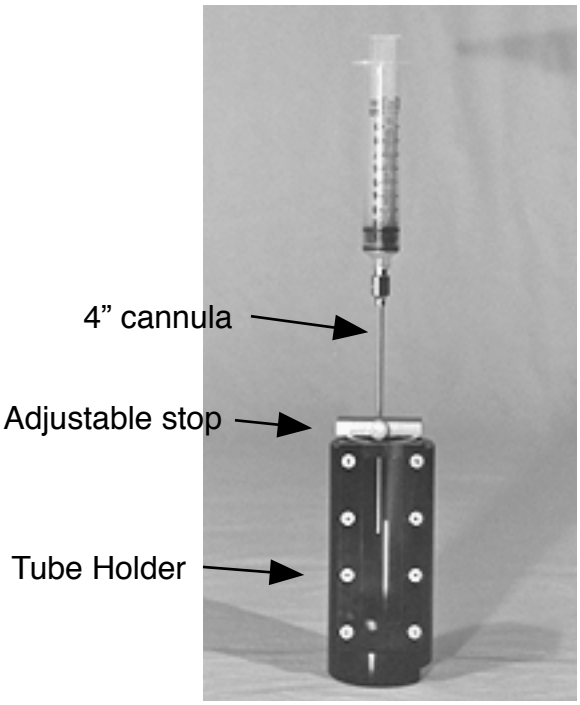
Place a small beaker under this tubing to catch the purge and then move the toggle valve to the DOWN position (the toggle points to the direction the water will take: UP is to the system, DOWN is to the purge line). Water may flow out the tubing without assistance, but if it does not, from the **MAIN MENU** press **FRAC** to get into the **Manual Menu** and then press the **RINSE** key to turn the pump on. A very brief pulse is enough to get it started.

Return the toggle to the UP position. Press the **RINSE** key again to observe whether rinse flows out the end of the collection tubing. No rinse should come out the end of the piston, whether the tip is attached or not. A check valve in the valve assembly prevents air or water from leaving the valve and disturbing the gradient while allowing the gradient to enter the valve during fractionation.

7.3 Setting the water level in the tube holder:

If you want to visualize your bands using the light scattering system, you will need to fill the tube holder with enough water to just cover the tube but not the cap when they are inserted into the holder. In the Accessory Kit, locate a 4" **water adjust cannula** with an aluminum bar attached to it. Connect this to a 10 cc syringe and fill the tube holder 3/4 full with water. Loosen the thumb screw and adjust the bar position along the cannula so that when the needle is inserted into the tube holder as far as the bar will allow and the barrel is pulled up to remove excess water, the water level inside the tube holder falls to a position where a centrifuge tube is just covered with water after insertion into the holder. Use an empty tube inserted into the Cap to set the needle depth and record the distance in mm from the tip of the needle to the stop for future reference. Water surrounding the outside of the tube will essentially make the tube disappear during fractionation, giving you an unparalleled view of the contents of the tube.

Omit this procedure if your particles are not visible by scattered light, and go directly to fractionation.



Insert an empty tube to see if the water level just covers it when it is fully inserted and locked

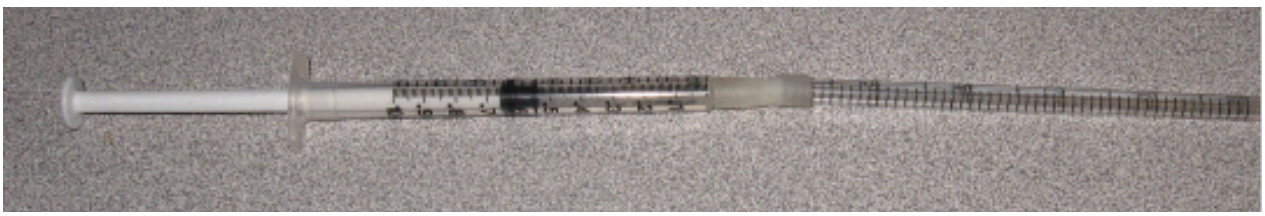
7.4 Sample layering for velocity runs:

If you want the highest possible resolution in your velocity (rate zonal) runs, you need to start with the thinnest possible sample on the top of the gradient. The highest resolution in rate zonal runs is obtained when 2-2.5 mm of sample is applied to the top of the gradient as shown below. If your sample is smeared or overloaded, the bands overlap and the resolution drops precipitously. Illustrated below is a simple layering device that outperforms automatic pipettors and pasteur pipettes, is inexpensive and reusable if needed.

Gradient preparation: First, form your gradients with the short caps using the Gradient Master and remove 0.1 ml less from the top of the gradient than the sample you plan to apply. If you are going to layer 0.3 ml samples, remove ~0.1 ml from one of the gradients you have just formed. TARE this first tube on a balance. Bring all the other tubes to within 0.05 g of the tared weight. This will allow you to load a constant volume of sample on the gradients and avoid having to rebalance the tubes before the run. When removing the top of the gradient, use the same layering technique illustrated below to avoid disturbing or creating a discontinuity in the gradient: pull the meniscus up the wall with the layering device shown and then suck off the required amount.

If your gradients will be run at 4°C, now is the time to put them well spaced and exposed in a refrigerator for 45 min to equilibrate at this temperature.

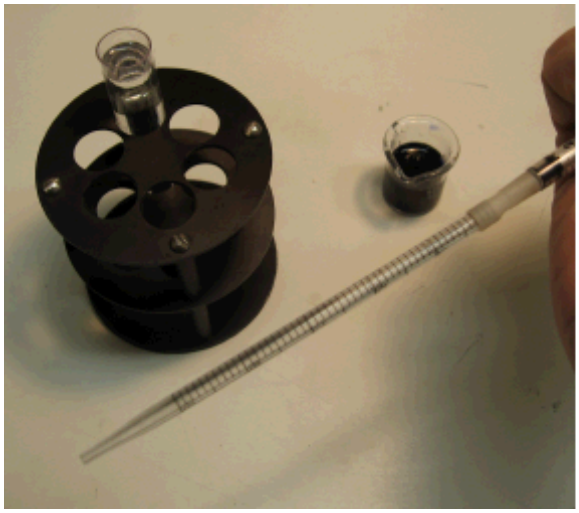
The layering device: For the SW40/SW41 tubes, take a sterile plastic 1 cc pipette and roll a razor blade over the 0.4 ml line to etch it, then snap the pipette in half at this line. Connect it to a 1 cc syringe with a short piece of flexible tubing (silicone is best) as shown below. Make as many of these as you have different samples to layer. Larger or smaller tubes are scaled with different sizes of syringes and pipets.

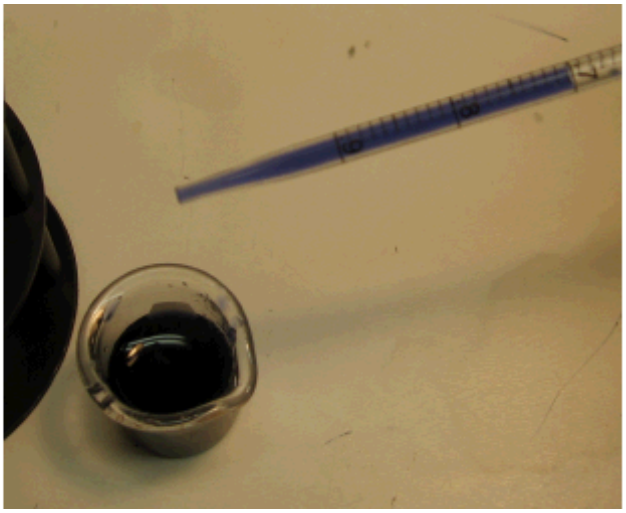


Sample preparation: If you pellet your cells, always resuspend your them in slightly more buffer than you plan to layer so you'll be sure to have enough of every sample. If you want to layer 0.3 ml, resuspend your sample pellet in 0.4 ml. When the cells are thoroughly resuspended and lysed in microfuge tubes, give them a 30-60 sec microfuge spin at 14,000 rpm to pellet all the large debris.

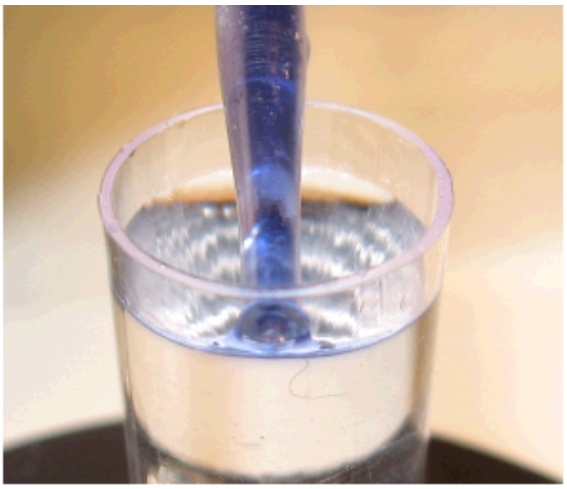
Sample layering: **CRITICAL: You must be at the centrifuge when you layer your samples.** Walking down the hall to the centrifuge with the already-layered tubes will ruin your runs.

Start by pulling 0.05 ml of air into the syringe to allow you to expel the last bit of sample when layering. Now, with the pellet on the high side of the microfuge tube, suck off the supernatant to the desired volume on the pipette (0.3 ml in the photos below). Leave no air at the tip of the pipette as shown: sample is flush with the end.

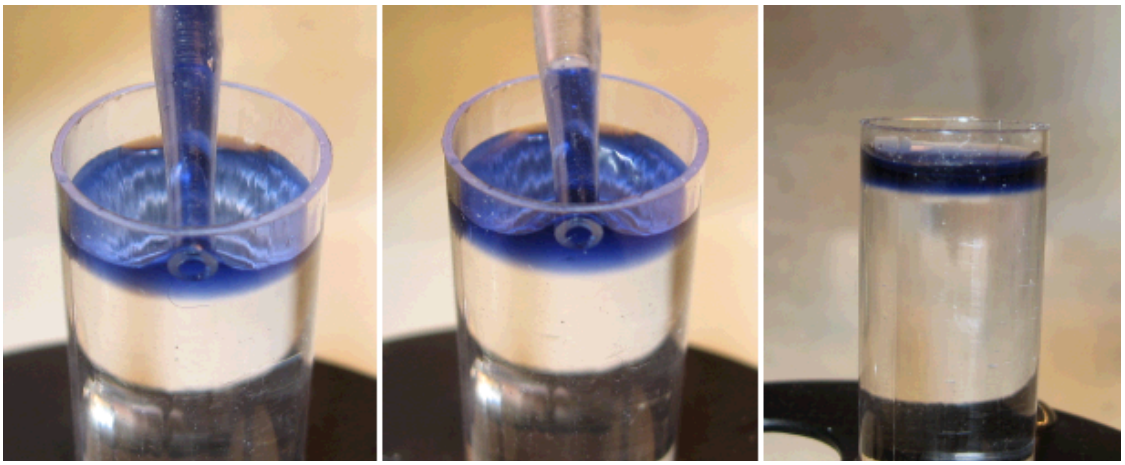




With the pipette nearly horizontal, insert the tip into the top of the gradient on the far wall and tilt the pipette to pull the meniscus up the wall.

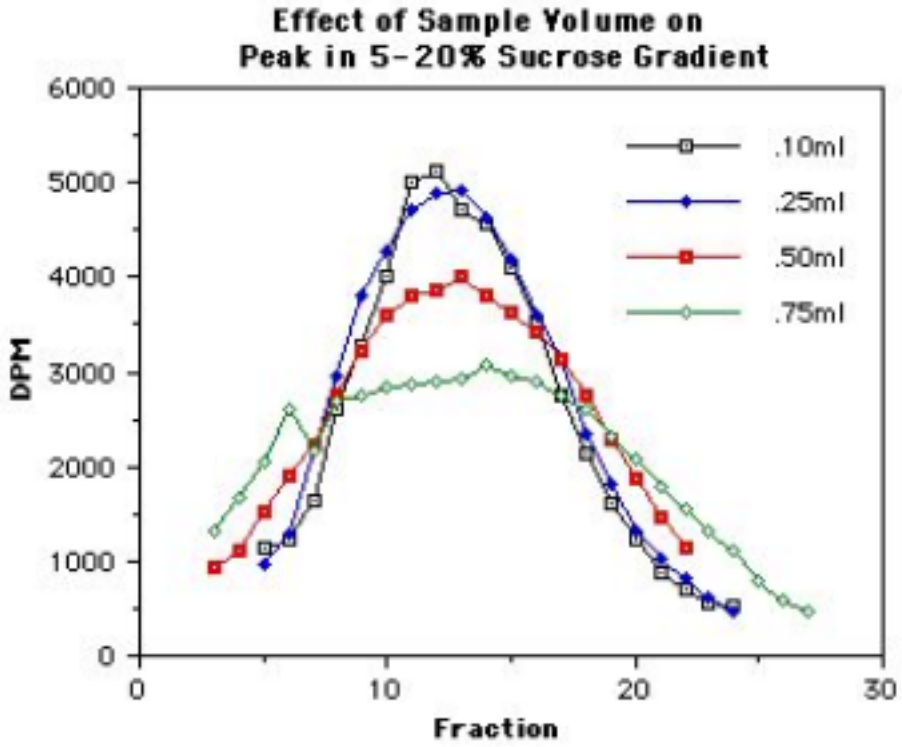


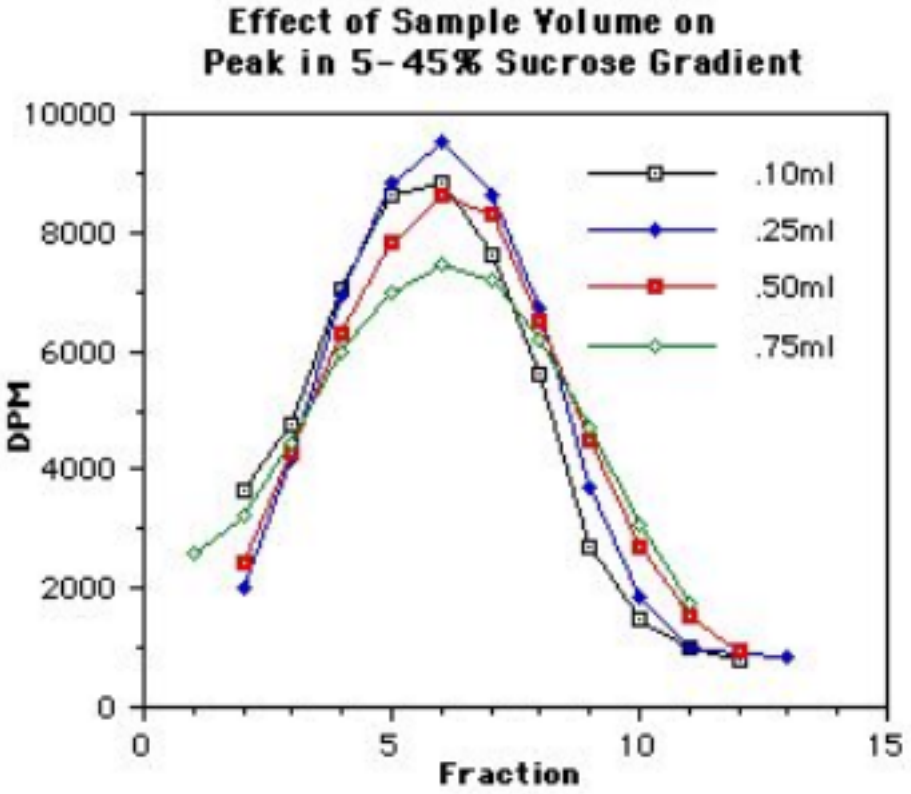
Start layering. Keep raising the tip up the wall to keep it above the level of the sample in the tube. When you are done and the last bit of sample has been expelled from the pipette, you are finished.



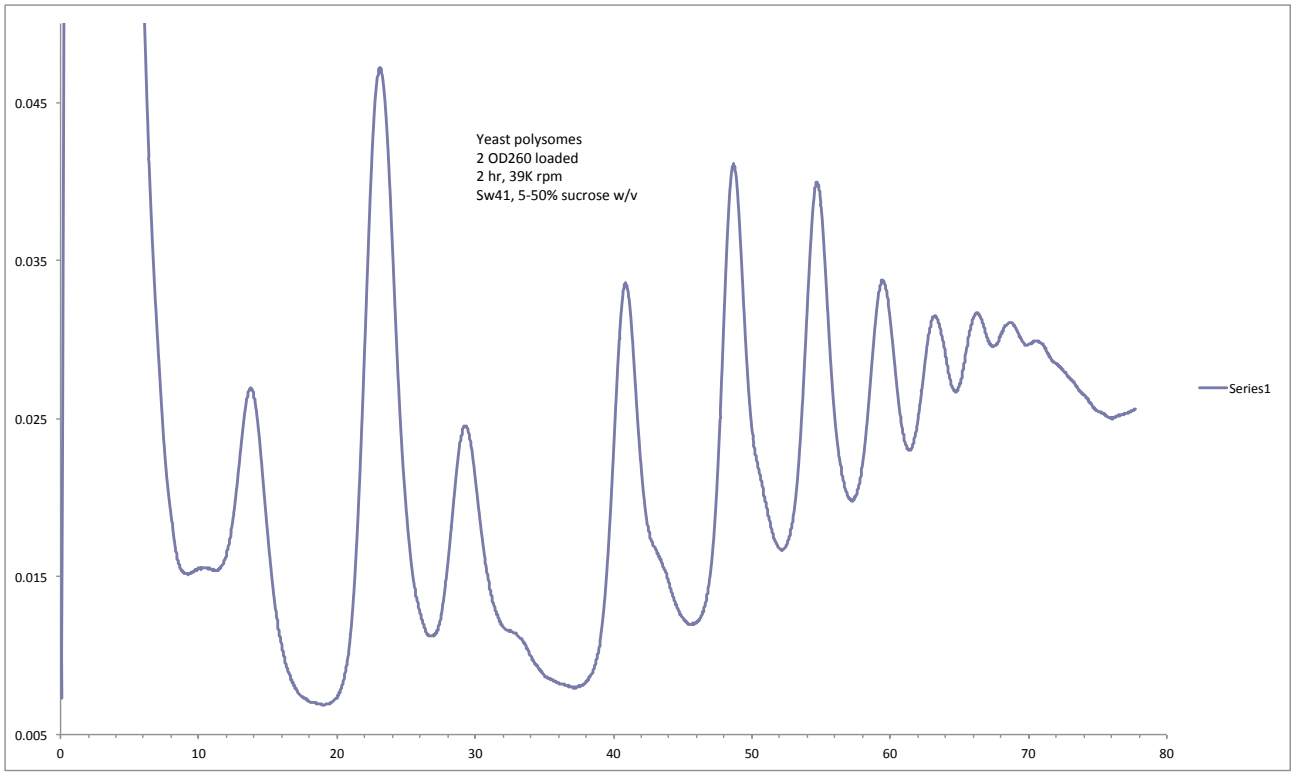
Tubes with different diameters will obviously have different sample capacities. As a general rule, your sample should be no more than 2.5 mm high to give the highest resolution. Samples that are higher than this will degrade resolution to some extent, depending on the endpoints of the gradient: Note that higher concentration bottom endpoints (>45%) preserve resolution.

7.5 Effect of sample volume on resolution: In the two gradients shown below, T4 phage were run about half way down an SW41 tube in the gradients listed and fractionated to observe the band resolution. The 5-20% run showed significant band spreading with the 0.5 ml sample, while the 5-45% run showed much less spreading with this same sample.





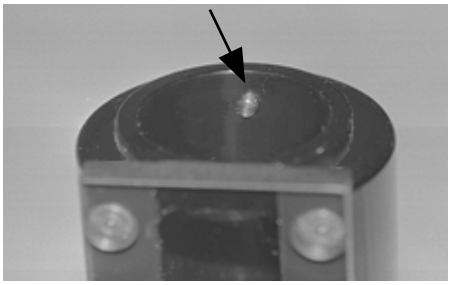
Polysome profile: SW41, 0.3 ml sample.

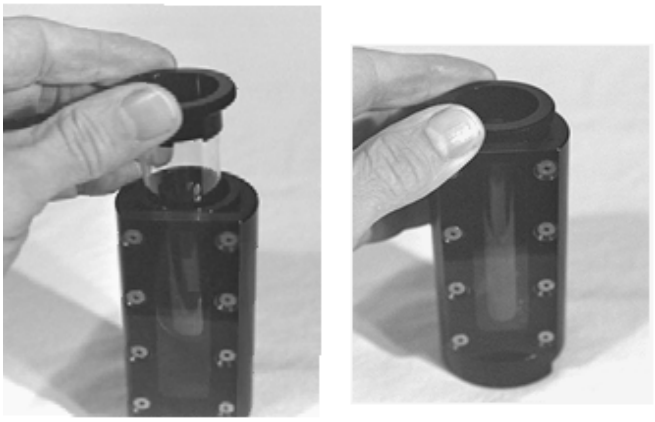


7.6 FRACTIONATING THE GRADIENTS

7.7 Inserting the centrifuge tube into the tube holder and locking it in.

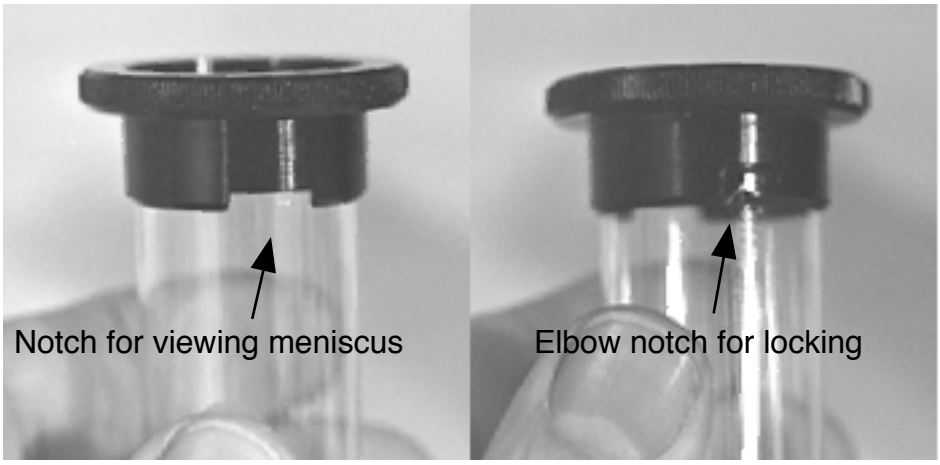
Locking pin (newer holders have 2 pins on opposite sides)





Place the tube in the holder and lock it with a clockwise twist

Take a gradient ready for fractionation and insert its upper end into the locking cap as shown below. You may find the cap in place in the tube holder. If the cap does not pull out of the holder, rotate it a few degrees counter clockwise to unlock it and then remove it. There is a flat notch in the front bottom rim of the cap that allows you to see the meniscus. The tube will sometimes catch on this notch instead of inserting fully. You can tell when this happens by observing the angle of the tube in the cap. It should be perpendicular to the cap if it is correctly inserted.

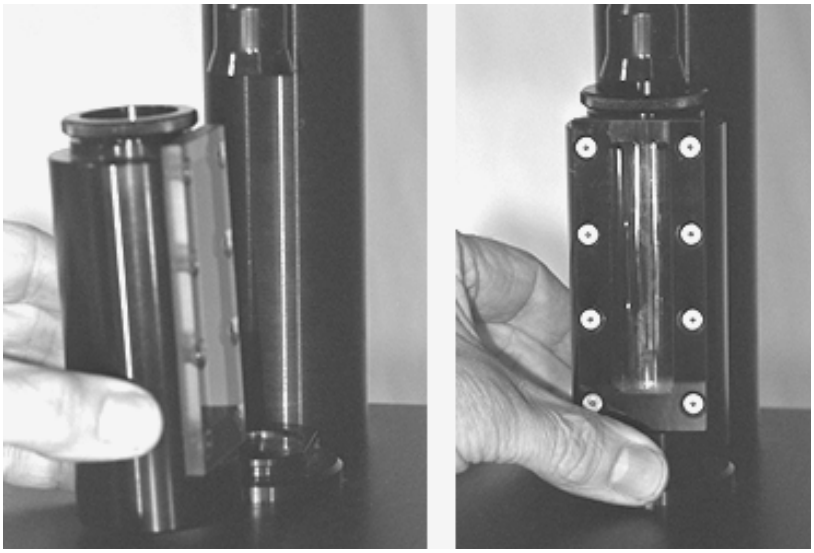


There should be enough "grab" between the cap and the tube to keep the tube from falling out as you insert it into the holder while holding only the cap. If you are not sure about this, pull lightly on the tube while holding it to see if it will stay. Use of tubes supplied by other manufacturers than SETON may lead to an improper fit between the tube and the cap with disastrous results.

Note that the tube holder has 2 opposing locking pins emerging on the inner rim. These pins fit in keyways on the side of the cap. After the tube and cap are inserted, rotate the cap until the pins are engaged in the keyways and the cap/tube slides all the way into the holder. Twist the cap a few degrees clockwise to lock the cap and tube in the holder. Pull up on the cap to see that it is properly locked.

7.8 Placing the tube holder on the fractionator:

Use the **UP** key to withdraw the piston so it clears the top of the tube holder. Turn the holder 90° so the window faces to the right. Slide the holder on the ring under the piston and turn it back 90° to lock it. Reverse this procedure to unlock it at the end of the run. The tube holder, cap and tube are now all firmly attached to the fractionator and will remain there when the piston is withdrawn without pulling up with it. You are now ready to fractionate.



7.9 Fractionation:

If you can see your bands, place the appropriate cursor (every tube size has a different cursor) on the window and mark the position of the bands and meniscus on tape using the sharp marker provided. Press **EXIT** to get to the **MAIN MENU**, press **RINS** and either scroll through existing rinse protocols using the **NEXT** key or enter your own rinse as described in section 6.4. Press **EXIT** to return to the **MAIN MENU** and then **FRAC** to enter the **Manual Fractionation Window**. Rotate the dial to bring the piston down into the tube. Always begin at the first drop described in Section 6.8 (pg. 18).

Decide how you want to fractionate the gradient (manual, macro, single or multistep) and proceed.

7.10 CLEANUP.

CLEANING IS A CRITICAL PART OF THE USE OF THIS INSTRUMENT.
IF THE TUBING AND VALVES ARE LEFT WITH SALTY GRADIENT SOLUTIONS OR RINSE IN THEM, THEY WILL RUST OR CLOG AND BECOME CONTAMINATED WITH MICROORGANISMS PRODUCING DESTRUCTIVE ENZYMES.

a. The tube holder and locking cap; mild detergent soak to decontaminate, but avoid **abrasive cleaners** or **organic solvents** that will damage the plastic window.

b. Tubes; since the piston does not puncture the tubes, they can be reused. In practice, tubes on their 4th or 5th cycle may collapse in the rotor under high G's, so 3 cycles is a safe limit. A big factor in tube collapse is water and solute in the bucket or on the outside of the tube. These may 'grease' the tube and allow it to slide down the swinging bucket. Always clean the swinging buckets and be sure they and the tubes are clean and dry before loading them.

c. The lower piston valve assembly; To clean the piston tip and valve assembly, fill a centrifuge tube with hot, soapy water, place it in the tube holder and manually fractionate it. Remove the soapy water by fractionating a tube containing only distilled water.

d. The rinse system and collection tubing; If buffer is in the reservoir, detach the syringe from the wing and discard the buffer by tipping the syringe into a beaker. Fill the syringe with **FILTERED,** deionized water, purge the pump by opening the toggle (down) and then pump water through the rinse system using

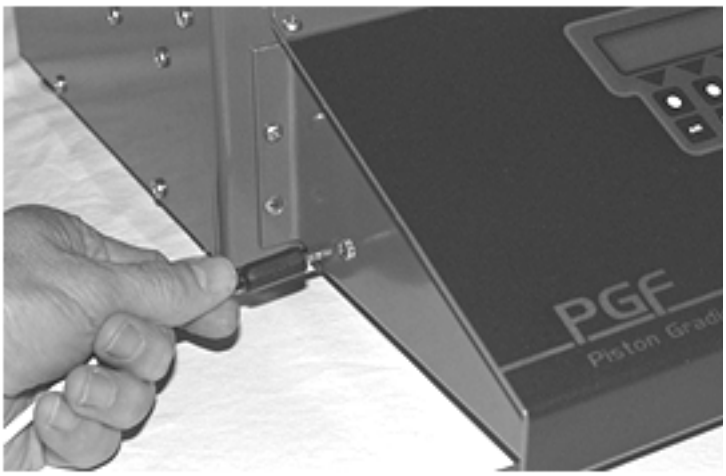
the **RINSE** key in the **Manual Fractionation Mode** (press **EXIT** 2 times and then **FRAC** to get to the **Manual Window)**. This will clean the upper valve assembly and the collection tubing. Since microbial growth can occur with only trace amounts of nutrients, it is important to disinfect the system before leaving it. Have a bottle of 70% ethanol ready. Pour out the water in the reservoir and replace it with a small amount of ethanol, prime ethanol into the pump (toggle), and then force it through the system with the pump. Finally, use the plunger on the 60 cc AIR syringe to force air through the entire system until no liquid comes out the end of the collection tubing. First open the purge line by flipping the toggle down and blow it out. With the toggle still down, pull up on the syringe plunger to fill it with air. Flip the toggle to **UP** and blow out the Rinse system. Flip the toggle down to refill with air and repeat. Do not use House Air to do this. It is usually full of rust and particulates that will clog the tiny check valves and is usually around 60 psi pressure, 4 X the pressure the system was designed to handle.

7.11 Connecting to a Fraction Collector

A cautionary note: A fraction collector has the potential to degrade the quality of the data produced because it requires a longer tubing connection than if you were to manually aliquot the outflow into vials held close to the piston. Laminar capillary flow, especially of viscous gradient solutions, sends a central core of rapidly moving fluid past the slower layers near the inner wall of the tubing, causing a smearing of the excellent resolution you achieved on the gradient. If you choose to use a fraction collector, place it on the left "wing" of the top plate as close to the piston as possible to minimize the length of tubing between the piston and the collector.

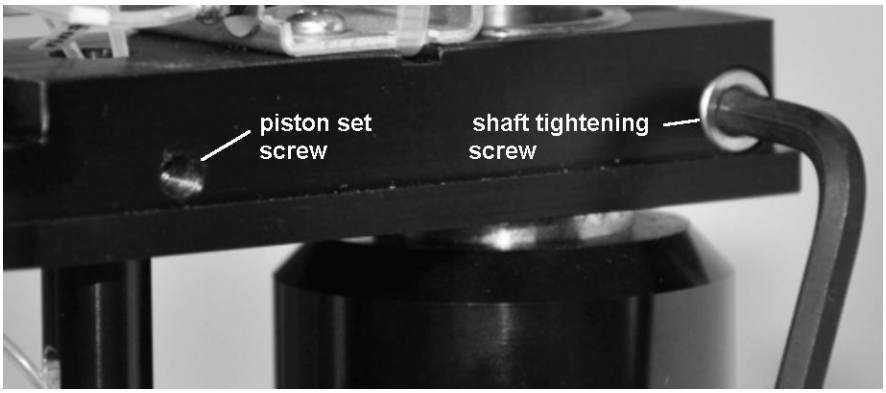
Find the Fraction collector cable in the kit box. The red wire is (+) and the black wire is (-) if your collector is polarized. Try the wires connected one way and if that doesn't work, reverse them.

The automatic connection from the fractionator to the fraction collector is made by a cable provided in the Accessory pack with a stereo jack on one end and red and black wires on the other. It plugs into a jack on the left side of the computer panel as shown. This is a contact closure source that most fraction collectors can recognize. When you press the **FRAC. ADV.** key on the keypad or call for an **'F'** in the programmed rinse, a contact is closed, allowing current from the collector to complete a circuit. The timing needed will vary with the collector, so try the shortest pulse (**'a'**)



7.12 Aligning the piston: side-to-side.

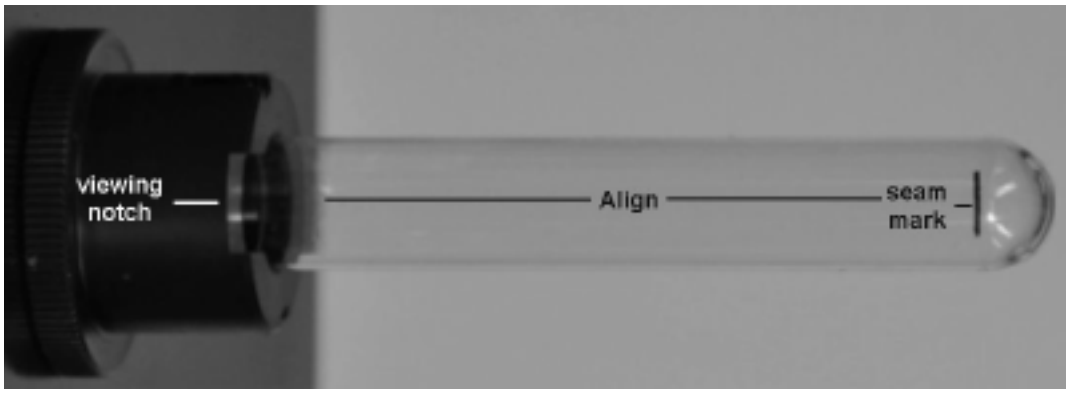
Side to Side: loosen the large cap screw on the right rear side of the nose piece (the round-ended piston holder shown below) just enough to let the nose piece rotate on the chrome shaft it is attached to. Lift the piston to the full up position, install the tube holder of your choice without the cap or tube installed. Screw the appropriate piston tip onto the end of the piston and lower the piston into the tube holder all the way to the bottom. Turn the light on under the holder. As you approach the bottom slow down and stop before the tip's seal enters the hole in the bottom of the holder. Move the nose piece left or right to center the seal in the hole. The ring of light around the seal should be even. Tighten the cap screw to lock the piston in the center position.



7.13 Setting the depth of the piston's stroke.

The cylindrical part of the tube above the hemispherical end is the only part of the tube that should be used for actual separations. As particles encounter the rounded end, they pellet and quickly slide to the very bottom of the tube. However, to harvest the last liquid from the cylindrical part, you need to lift it up into the valve where it can be captured and harvested. This means putting the piston slightly below the seam into the round end. Follow these steps carefully to set the depth of stroke **no deeper than 2 mm into the round end**.

1. Draw a thin line on the seam between the tube's wall and round end as shown. Align the mark with the viewing notch on the cap and insert the tube/cap into the tube holder. Place the assembly in position under the piston and move the piston down into the tube using the knob.



2. Keep going until the piston reaches the lower limit switch and stops by itself. Press the EXIT key a few times until you return to the main menu (FRAC RINS STUP). The press FRAC and RESET to 0.00 the distance on the display.

3. Turn the dial to full CCW and press the UP key on the top row of the keypad. This will make the piston to start upward. It will only take a few seconds to go beyond -2 mm, so when you are past that mark, press the DOWN key to stop the piston. Now turn the dial slowly CW to move the piston down so that the seal's bottom edge **lies directly over the seam mark**.

4. Use the 3/32 allen wrench in the kit (third from smallest) to loosen the set screw that holds the piston on the right side of the nose piece as shown below. The piston will remain at the align mark while the piston holder now retracts

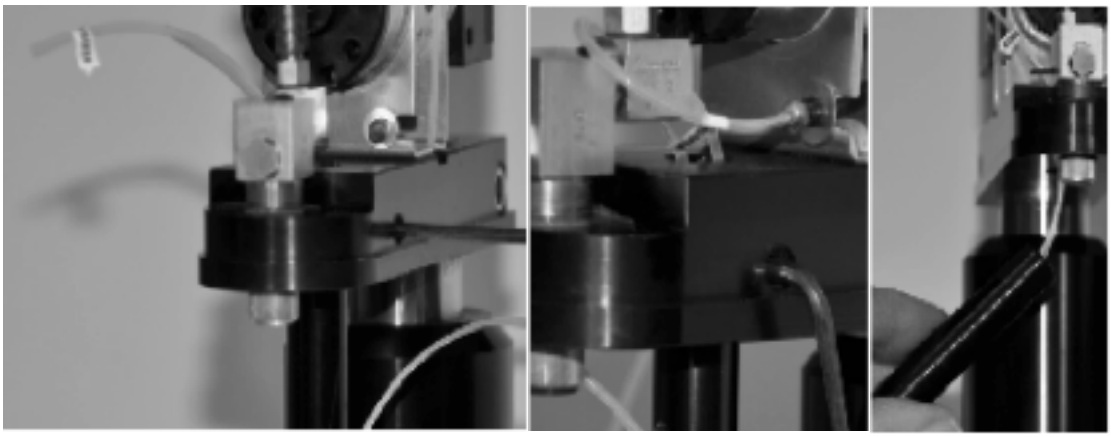
5. Press the UP key to move the nose piece up a few mm and then press the DOWN key and slowly move it down to -2 mm. The piston itself should remain at the seam mark.

6. When both the objectives have been achieved (seal at mark, mm at -2), tighten the set screw to lock the maximum depth of your stoke to 2 mm below the seam. Try to lower the piston to the bottom limit switch to make sure it is able to reach the switch. If it fails, set the depth to a smaller value, i.e. -1.5 mm.

7.14 Swapping the main piston/valve and the 11 mm piston/valve.

The main piston/valve assembly is removed as a single piece and replaced with the 11 mm piston/valve.

1. Remove the EM-1 bracket and the red tower cover.
2. Then remove the Air and Rinse tubing from their respective needles as shown in Section 8.4 below.
3. Loosen the set screw on the right side of the nose piece (left figure below) that holds the piston in place and pull the piston straight down, tubing and all.



Disconnect the rinse and air tubing Loosen the piston set screw Pull the piston down

4. Insert the 11 mm piston, tubing first so that the Air and Rinse tubing exit the top hole aimed to the left as shown in the left figure above . Make sure the Sample tubing hole faces front.
5. Tighten the set screw while turning the piston back and forth to make sure the flat faces the set screw.
6. Reconnect the tubing as shown in Section 8.4 below.

SECTION 8. MAINTENANCE AND REPAIR.

8.1 Lubrication:

The only part you can lubricate is the chromed **Push Tube** the piston carrier (nose piece) is attached to. At the back of the black **Bushing** that guides the shaft there is a grease nipple that fits any standard grease gun and a screw below it. On an annual basis, or more often if it is used heavily, remove the bottom screw and attach the grease gun to the nipple with the piston in its full DOWN position. Give it 3 strokes of the gun and reapply the screw.

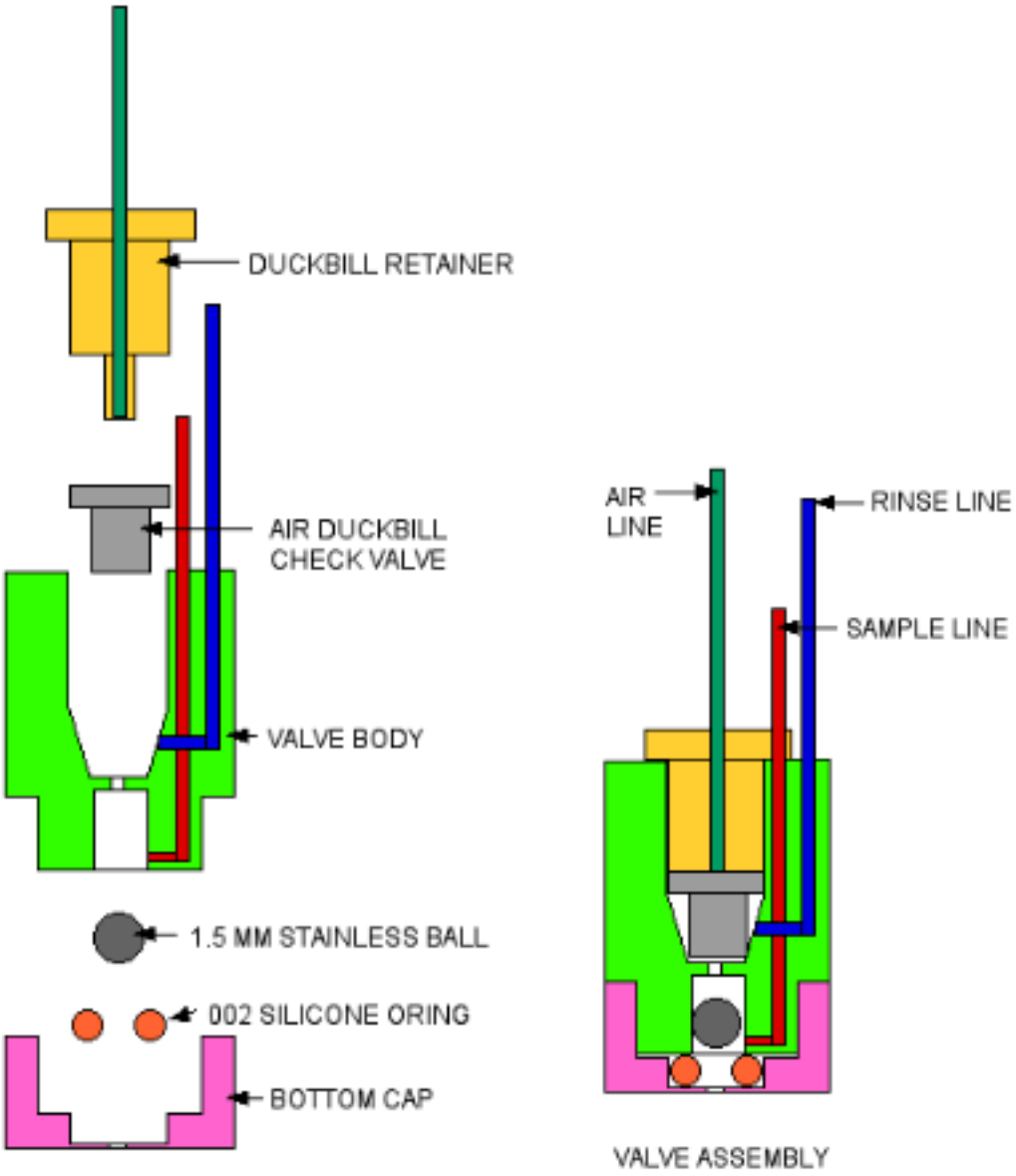
Keep the exposed shaft of the Push Tube clean and free of water by wiping it dry with a soft cloth and applying a light coat of lithium or moly-based grease to the exposed shaft when the piston is in its full UP position.

The air pump on the top of the piston carrier is a reciprocating diaphragm type and it has an eccentric cam that drives a piston. Have a technician inspect and lubricate the cam yearly. Contact BioComp for replacement diaphragms should they be needed.

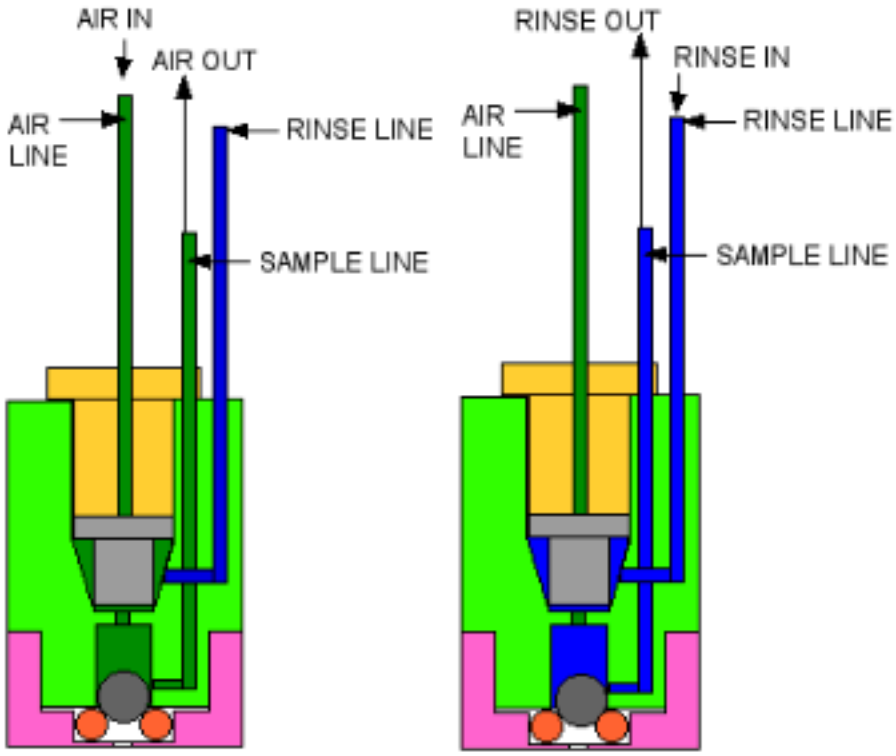
The water pump and stepper motor are permanently lubricated.

8.2 Normal valve function.

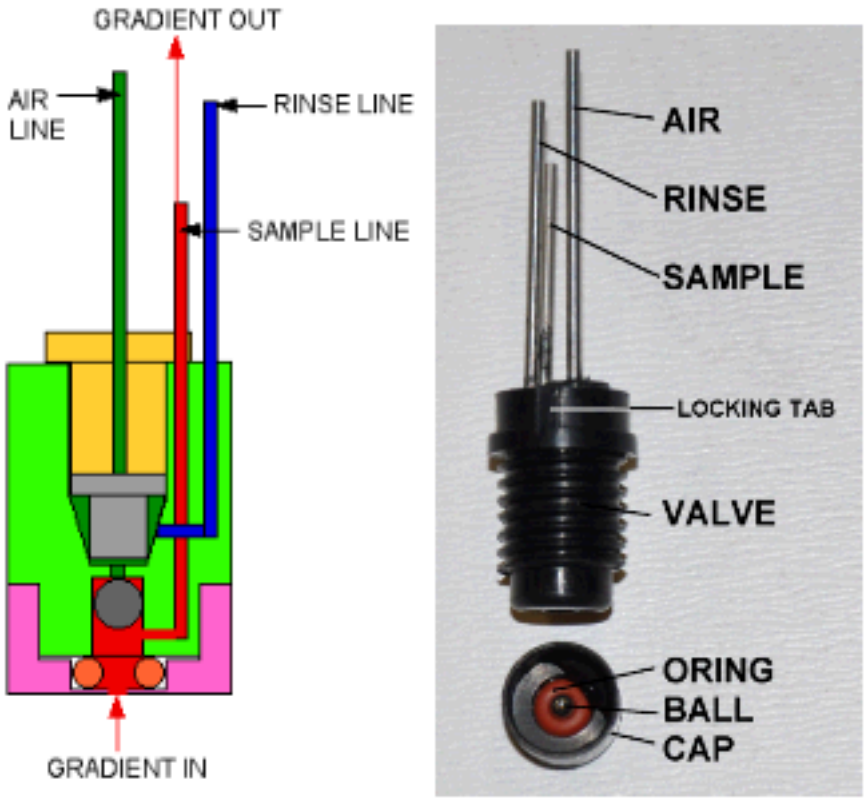
In order to understand how the valve works, view the assembled and blown up diagrams below and follow the narrative that follows:



The **air flow** is shown in the left figure below. The **rinse path** is shown in the right figure. In either case, the ball is driven onto the ring below it, sealing off the gradient from rinse or air..



When the piston is moving down into the gradient, the inflowing liquid lifts the ball off the oring and the gradient exits up the **sample line**. Check valves prevent the gradient from entering the air and rinse lines.



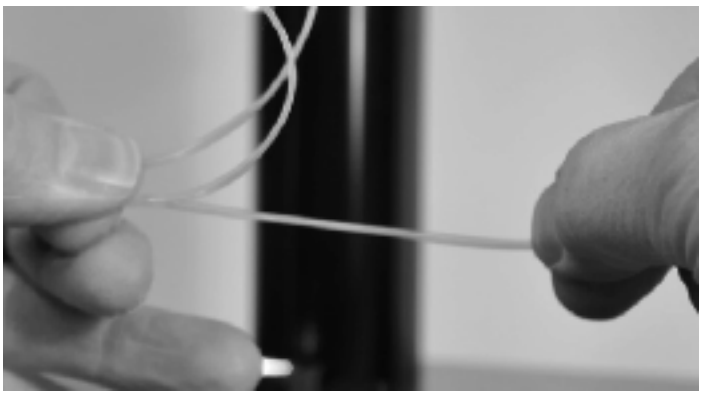
8.3 Valve replacement and maintenance.

There are several reasons to remove the valve from the piston: to change it for the spare, to test its function and to replace the tubing. The 3 pieces of 1/16" OD Teflon tubing (**45 mm** for air, **75 mm** rinse and **450 mm** Sample) in the piston require occasional replacement, especially the sample tubing, which is exposed to membraneous material.

To remove the valve:
1. Remove the red tower cover on the nose piece (piston carrier) and detach the air and water tubes from their needles by pushing back on the very end of the tubing with your fingernail as shown below. This expands the tubing and removes it without stretching it.
2. Unscrew the piston nut that holds the valve in the bottom of the piston as shown in the center below.
3. Pull the valve and associated tubing down and out of the piston as shown to the right.



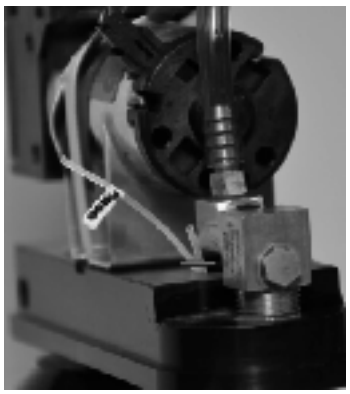
4. To straighten out the coiled sample line on a new valve, pull it through your fingers while curving it opposite to its natural curve. It is like curling a ribbon.



5. Feed the sample line up into the piston until the end emerges from the hole near the top of the piston as shown.

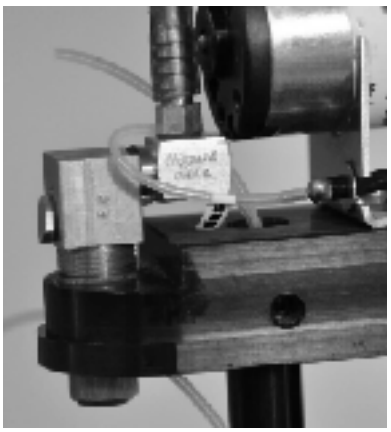

6. Notice that the air and rinse tubing are oriented facing away from the sample tubing. This eases the insertion. As you feed them into the piston, keep their curl facing the left inner wall of the piston. Push them in, avoiding the sample hole and tubing until they emerge from the hole on the top of the nose piece as shown to the right below. They must emerge on the **left side** of the nose piece.



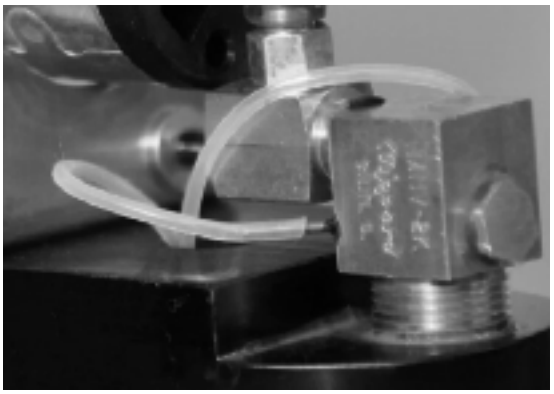


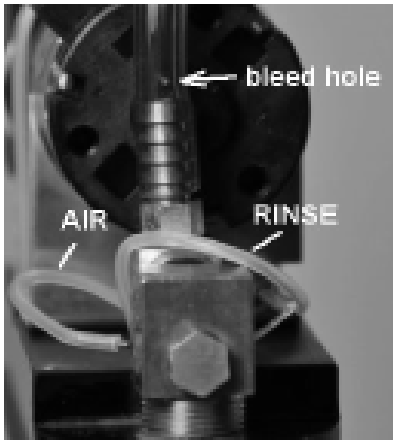
7. As the valve approaches the tip of the piston, align the tab above its top ring with the notch in the bottom of the piston as shown. This is critically important, since the tab is the only thing that keeps the valve from turning as you unscrew the piston tips. If the tab fails (usually from over tightening the tips), there is no way to remove the tips from the piston. **Apply the piston nut as shown and tighten it, again avoiding over tightening as it is very thin plastic and can be broken by over tightening.** You can tell if the nut is tight enough when the valve is held firmly in the piston.

8. Now connect the tubing to the appropriate needle: Rinse (marked with its label, it is the longer of the two) to the needle on the right side of the nose piece under the air pump and Air to the needle on the left side attached to the brass air needle valve as shown. The lower right view is how the tubing looks from the front of the nose piece.

This is how the tubing should look:





9. To simply replace contaminated teflon tubing, remove the piston nut, remove the rinse and air tubing from their respective needles in the tower and pull the valve down and free of the piston as described above.

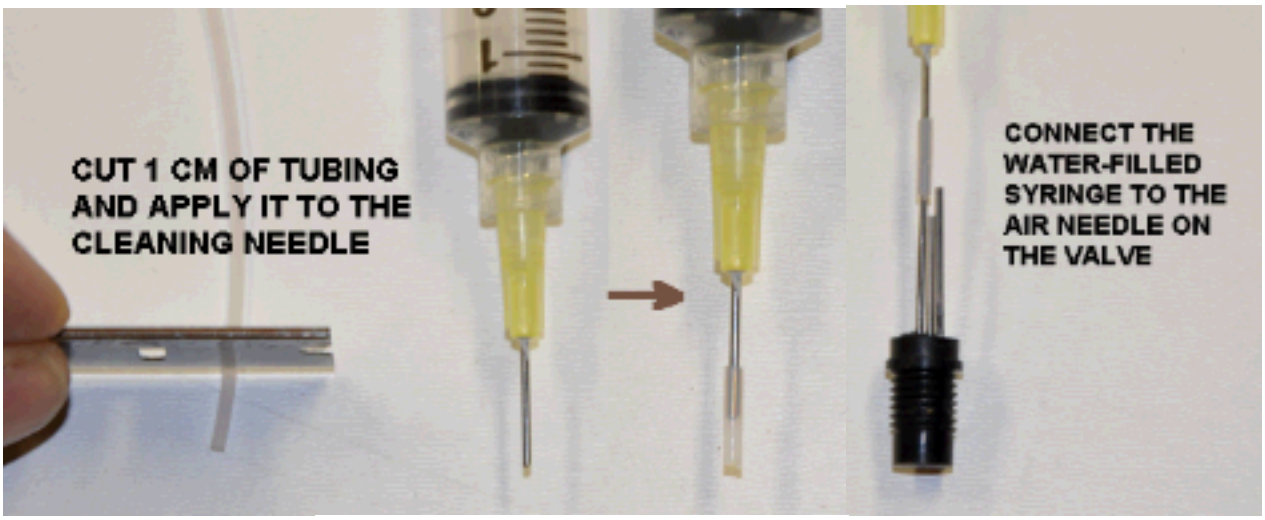
10. Cut fresh tubing of the same length (**45 mm** for air, **75 mm** rinse and **450 mm** Sample) from the spare roll in the kit. Use a sharp pointed object to expand the open ends of each piece of tubing and then insert the cleaning needle (20 ga) to expand the ends to facilitate assembly on the piston needles. Push the tubing 4-5 mm over their respective needles on the valve assembly. The orientation of the lines looking down on the top of the valve assembly is as follows:

12. To reassemble the tubing system, follow the instructions in section (4) above. Label the rinse tubing **"R"** so you can tell which is which. The needle in the brass needle valve is the AIR and the needle under the air pump is the RINSE. Replace the red tower cover.

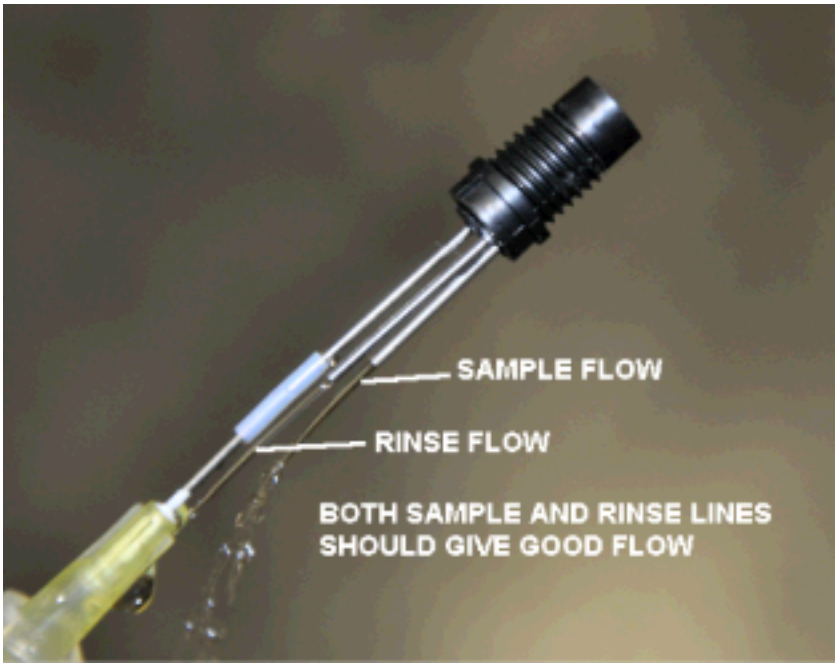
8.4 Valve performance testing.

1. Remove the valve from the piston by removing the red tower cover, disconnecting the Rinse and Air tubing from their respective needles, loosening the nut that holds the valve to the end of the piston and pulling the valve down and out of the piston. See section (4.) below for details.

2. Prepare the cleaning syringe by applying 1 cm of teflon tubing from the kit to the cleaning needle and 10 cc syringe and fill the syringe with distilled water. Cut a short piece of teflon tubing from the coil in the kit and attach it to the cleaning needle as shown. remove the valve's tubing and connect the cleaning needle to the Air needle

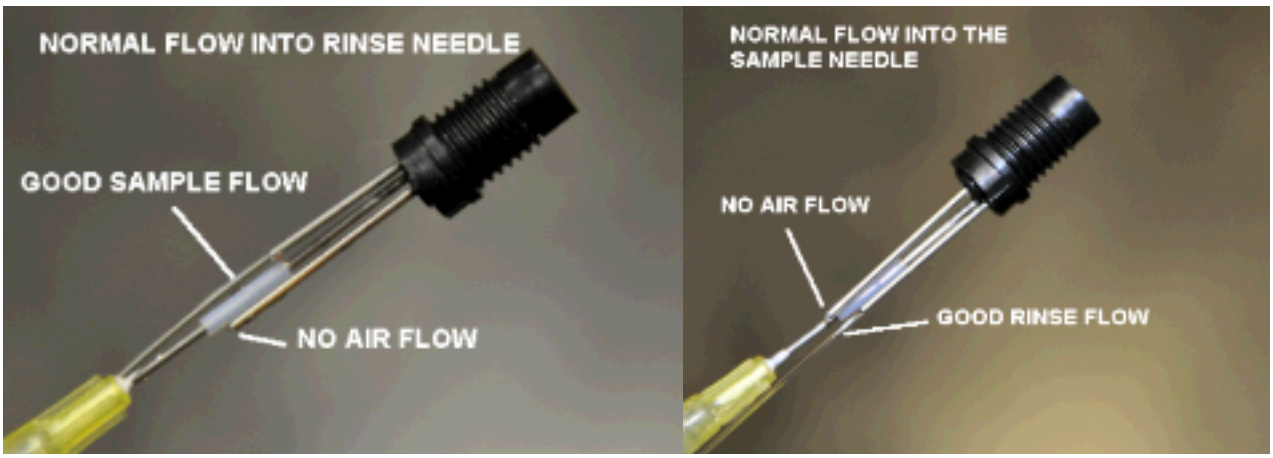


3. Force water into the Air line and observe the flow. The normal valve gives solid flow through both the rinse and sample lines. If either is clogged, its flow will be reduced or stopped in that needle.



4. Connect the syringe to the Rinse needle. Here is normal flow when water is forced through the Rinse needle. If the Rinse line is clear and the Air check valve inside is working, only the Sample needle should give any flow. Any flow out the Air line indicates that the air check valve (the rubber duckbill) has a particle in its slit keeping it from collapsing and sealing.

5. The same logic applies to the Sample needle: only the Rinse needle should show any flow.



8.5 Valve repair

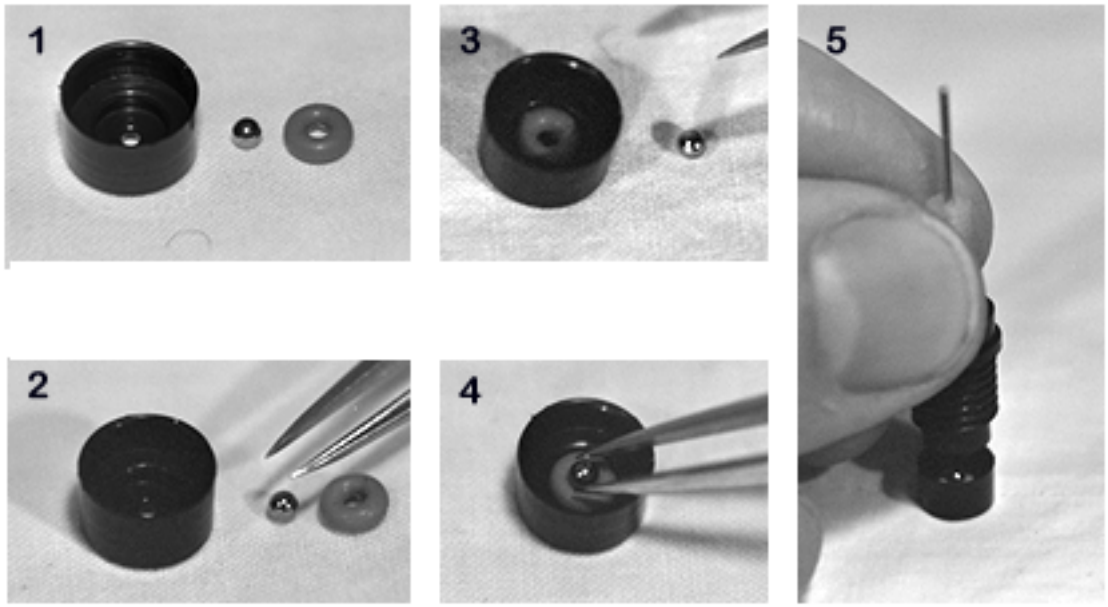
1.**Ball valve in bottom cap is clogged or leaks during rinsing or blowing**. At the bottom of the valve assembly where the piston tips screw on is a friction-fit cap containing a small silicone oring and a tiny stainless steel ball. These act as a check valve, preventing air and rinse from entering the tube, but allowing the gradient to flow upward as the piston moves down. When the piston tip is screwed on, it forces the cap and the oring to contact the upper valve body, sealing the cap.

If the valve itself is leaking out the bottom hole or you find a clog, remove the cap on the bottom of the valve and make sure you capture the ball inside on a wet paper towel. Pull the cap straight down off the

valve. Avoid rocking it side to side as you remove it as this will expand it and loosen its grip on the valve. See the photo in 8.2 above

Two kinds of problems are possible with the cap: clogs and leaks. Clogs are easily seen and fixed. If all the lines pass the tests below but the flow of gradient up into the valve is blocked or impeded, you can be certain that the ball valve is clogged. Disassemble and examine the ball, oring and hole in the cap for obvious blockage. Another possibility is that someone added a ball to the cap without seeing that the old ball was retained in the valve body itself. If you look at the ball cavity in the valve body, it will be obvious whether the ball is there or not.

Leaks in the ball valve are more common. They arise when the ball does not sit evenly in the center of the oring during rinsing or blowing. Typically, the ball or oring are dirty or the oring is distorted or has irregularities in its upper surface that prevent the ball from sitting in it and sealing.

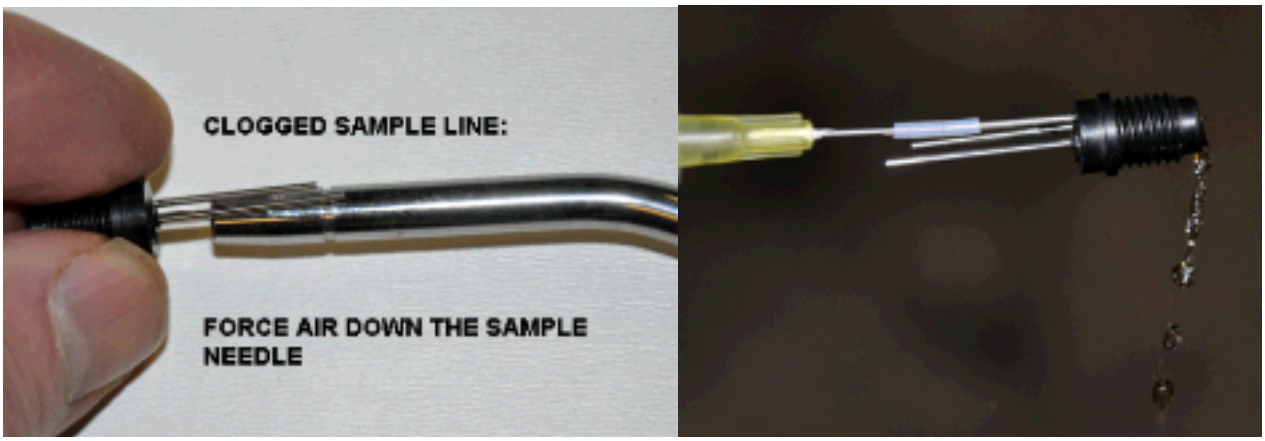


BEWARE THE ROLLING BALL!!

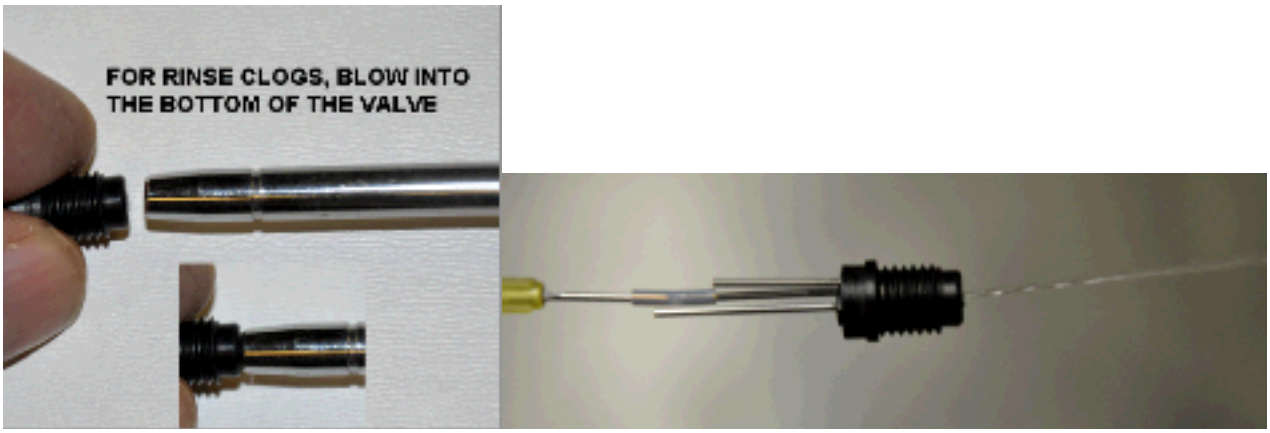
The stainless ball is very small and will roll a very long way if you drop it, so only perform this maintenance over a wet paper towel that will prevent the ball from rolling away. Have a pair of fine tweezers ready to handle the ball and the oring. Use hot soapy water to clean the ball and the oring, and an organic solvent on the ball if soap does not work. The oring should not be put in organic solvents. Examine both in a dissecting microscope to see that they are clean and the oring's surface is smooth.

Before you reassemble the ball valve, connect a water-filled syringe to the sample tubing and force water backwards into the Sample Tubing toward the valve to be sure that there is no obstruction inside the body of the valve assembly. Place the oring in the center of the cap (3 above), place the ball in the center of the oring (4 above) and carefully press the valve body **straight down** into the cap. Test as described above. Liquid and air should not flow backward through the ball valve when you force air or rinse through their respective needles or press the RINSE or AIR keys in the manual mode.

2. **Clogged Sample line:** with the cap removed, blow in the opposite direction to the normal flow through the needle. The gradient normally flows up into the sample needle so the idea is to blow down the needle to remove the clog. Locate a source of clean, high pressure air such as a spigot on the lab bench. Insert the sample needle (the shortest needle) into the spigot and turn on the air full blast. Connect the cleaning syringe to the needle to see if water can now pass. With the cap off, a clear Sample line gives a lateral spray out the bottom of the valve, as shown below.

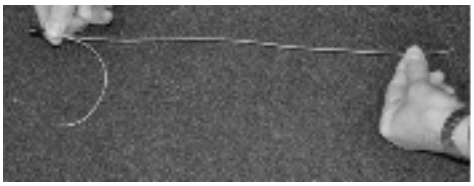


3. **Clogged Rinse line:** Since Rinse flows down the needle into the valve, blow into the bottom of the valve to reverse the direction of flow. Connect the cleaning syringe to the Rinse needle (the middle length needle) and force water down into the valve. A fine thin line of water should come out the center of the bottom of the valve. Water injected into either clean Air or Rinse needles exist through the small hole in the center of the ball pocket, giving a smooth straight stream of water.



4. **Leaking Air check valve**. In the very first test above (8.3.3), when you force water down through the Air needle and then pull back, no air was supposed to aspirate backwards into the syringe. If the duckbill valve has particles stuck in the slit at its mouth, rinse and sample will back up into the Air line during fractionation and you will be able to pull air into the syringe connected to the Air needle. There is about a 50% chance of fixing this type of leak. The idea is to remove the particle with high pressure air or water. Try forcing air or water down the Air needle as shown for the clogged Sample line above. For water, use a 1 cc syringe connected to the Air needle and force water into the needle as hard as you can. Failing this, connect a 1 cc syringe filled with distilled water but lacking its plunger to the Air needle and blast the syringe full of water through the duckbill **with air pressure**. If none of these methods work, the valve needs to be replaced.

The ultimate, low pressure leak test for the Air duckbill check valve is to connect a long piece of teflon tubing to the Air needle, fill it with water using the syringe, and gradually invert the valve, with the tubing below to create a small hydrostatic head. If the duckbill is working, no drops will fall from the end of the tubing.

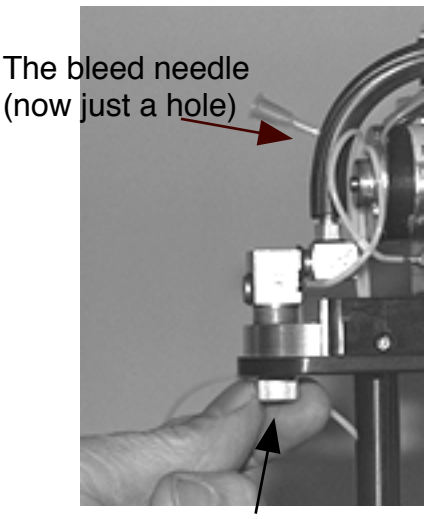
UP  DOWN

4. Final Check. Before reassembling the valve inside the piston, check its function. The bottom cap with its ball valve (see above) must be assembled to do this. Connect the tubing to the 20 ga cleaning needle (supplied in the kit) and a 5 cc syringe filled with water. Connected to each of the three lines (one at a time), the syringe should behave as follows as you push or pull the plunger on the syringe:

Air Line	Push	Water should flow out of both Sample and Rinse lines but not out of the ball valve at the bottom.
	Pull	The syringe should pull a vacuum. No water or air should travel up the air tubing into the syringe.
Rinse Line	Push	Water should flow out of the Sample line but not out of the Air line or the ball valve.
	Pull	The syringe should aspirate air easily, but should have difficulty pulling up if the valve is immersed in water.
Sample Line	Push	Water should flow out of the Rinse line but not out of the Air line or the ball valve.
	Pull	The syringe should aspirate air easily.

8.6 Adjusting air pressure:

The air pump is capable of 15-18 PSI but only 3-6 PSI is needed for most fractionation. Thus there must be some way of limiting the air pressure. This is the reason for the needle valve just in front of the piston in the piston carrier. The brass knob screws up to limit the flow of air and down to increase it. Even with this feature, the pump would rather not work against high back pressure, so there is a small needle or hole in the black tubing that connects the pump to the needle valve to dump some of the excess air. If the range of air pressures offered by the needle valve is too high for your needs, you can increase the size of the hole by inserting a large syringe needle into the tubing.

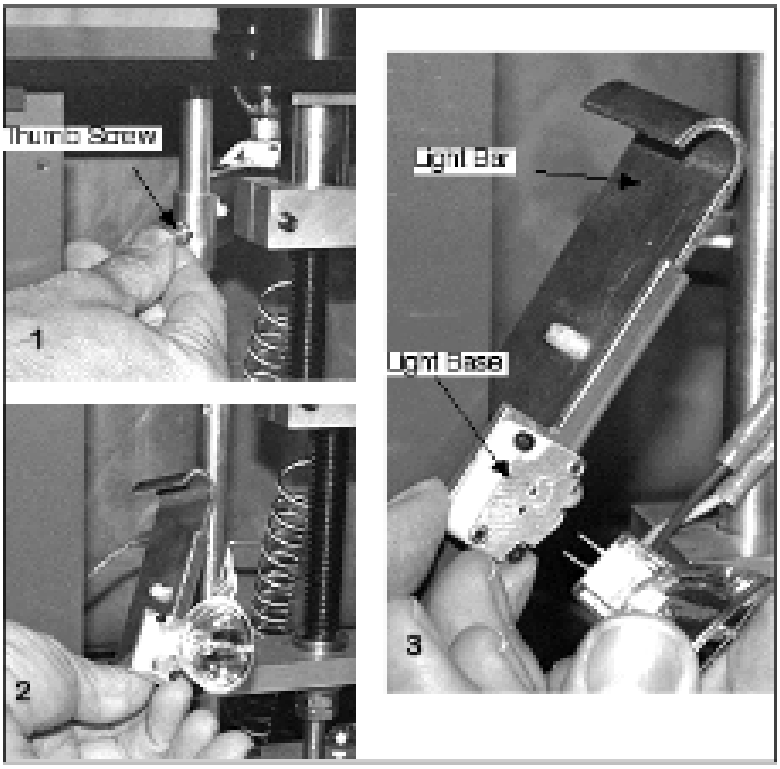


Adjusting the air flow

8.7 Changing the bulb.

The **HALOGEN SPOT** under the top plate will eventually have to be replaced.

Older PGFs and Stations: This is accomplished by unplugging the fractionator and removing the 6 screws from the back panel and the brass thumb screw holding the **LIGHT BAR** to the rod. Slide the Light Bar out of the unit where you can remove the bulb by pulling it out of its base. Locate the replacement bulb in the kit and insert it's pins into the light base and reverse the assembly.



PGFs and Stations from 2013 onward: Undo the 4 screws holding the top front panel and remove the panel. You will see the halogen light positioned below the piston. Unscrew the single philips screw holding the light base to the light bracket, tilt the base to the left and remove the bulb as above. Reverse to install the new bulb.

8.8 Piston Tips and Seals: We initially designed the black PVC piston tips seals to be replaceable. Newer silicone tips should last many years. The seal is itself a one-way valve; forcing liquid up through the center of the piston on the down stroke but letting air into the tubes on the up stroke.

PVC black seals: The sharp edge of the seal is fragile. When it will fails, gradient solution leaks into the space just above the seal. **Watch the volume of water above the seal on the outside of the tip**. As the piston descends, this dark area should grow only slightly. It would be an excellent practice to put a water-filled tube in the holder before beginning a run and forcing the piston down into it manually just to see how it behaves.

PVC Seals:
a) Things to avoid:
hot water (heat of any kind) - tends to shrink the seal
organic solvents - removes the plasticizers
petroleum-based greases

b) Things to look for:

PVC seals: Nicks or folds in the seal's leading edge that cause leaks. Use a dissecting scope once in a while to inspect the seal's lower edge for defects.

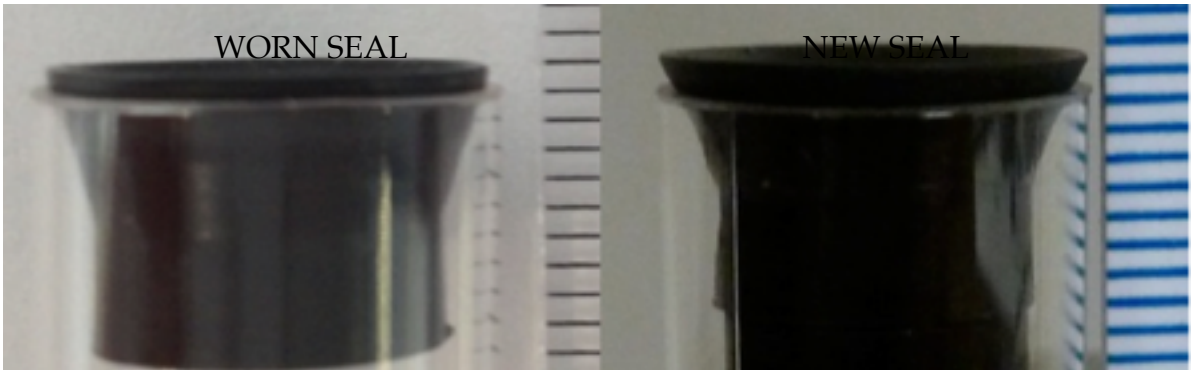
Silicone tips resist heat and solvents, and can be deformed without damage.

Tubes with obvious defects that can grab the leading edge of the seal and bend it back

Tubes with deep grooves down the seams in the side walls (look at the obvious seam on the bottom of the tube to locate the seams in the side wall). These tubes can leak, though they do't affect the seal.

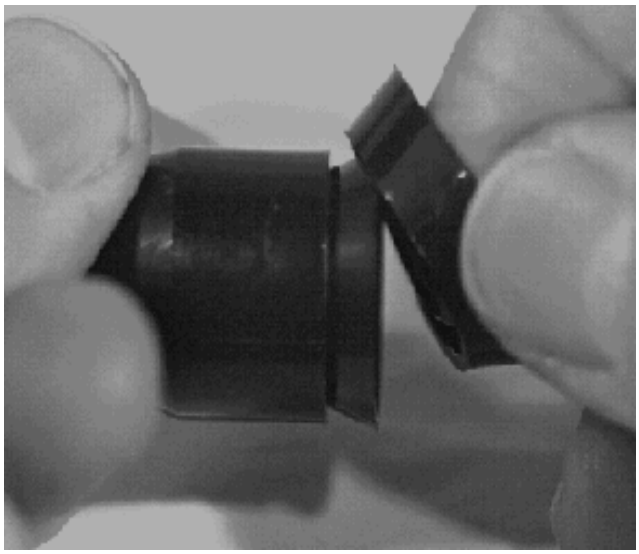
c) Finally: The tips can contain a bit of heavy solution from the previous gradient. When you start fractionating the next gradient, this heavy solution can plummet down the length of your new gradient rendering it useless, so it is important to remove the tip from the piston and rinse it out between runs. Better yet, have all your tips ready and just swap them out after use.

8.9 Replacing the PVC piston seals: There comes a time in the life of every seal that it needs to be replaced. There is a simple test you can do to determine if the seal is still worth using. Take a new tip and the tip you are using and invert them in the top of a tube as shown:



The used tip on the left sits further down in the tube and its seal's outer diameter is essentially the same as the *inner* diameter of the tube. The new seal on the right sits higher and has a diameter that matches the *outer* diameter of the tube. Since the seal can only work if it is compressed against the inner wall of the tube, it is obvious that the used seal on the left needs to be replaced.

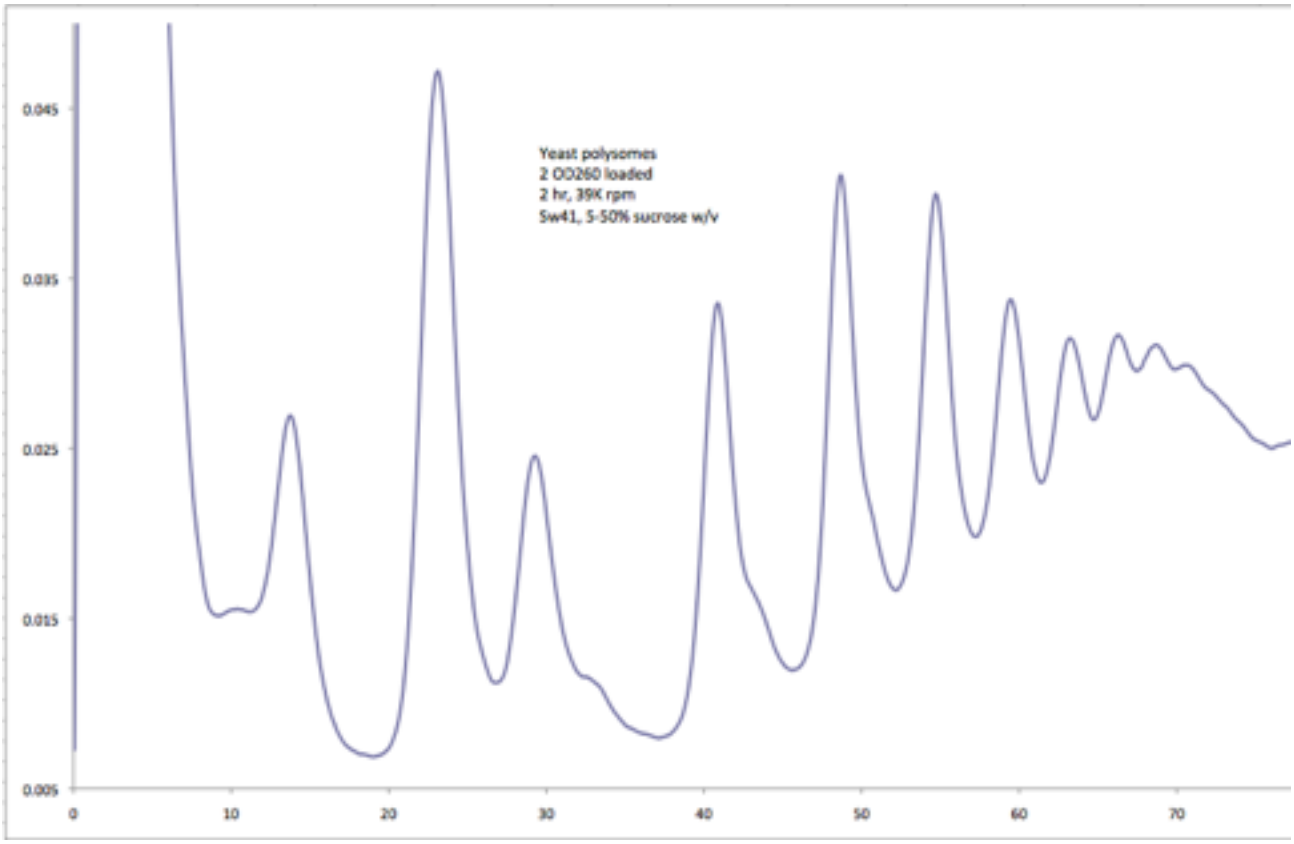
Replacing the seal involves pulling the old one off and fitting the new one on in its place as shown below. **Always immerse your seals in warm (not hot) water before removing or applying them to the tip.** Use rolling pressure to seat the seal on the tip, that is, don't rotate the seal on the tip but apply pressure in a rolling fashion. When the seal is on, press around the upper ring to force it into the groove in the tip to even out any tension. Quickly look inside to make sure that the seal's upper, inner edge where it meets the hard tip's trumpet is not tucked under. Release any tucks by bending the seal away from center at the point of the tuck. Before using a new seal, always test the fit for leaks by fractionating a water filed tube.



8.10. Continuous flow UV-based fractionation

We are currently adapting the BioRad Econo UV monitor, EM-1 flow cell by connecting it directly to the piston fractionator's sample line. If you are considering purchasing this unit, contact us and we can supply you with a special mount that permits you to attach the flow cell directly to the red tower cover.

The yeast polysome profile below was obtained with the EM1 flow cell.



Contact us for details on the system and its use.

8.11. Miscellaneous:

- Always remove the piston tip at the end of each run and rinse it out to remove the heavy last fraction from the previous gradient. Check the inside of the threaded end of the tip. It should be dry. The presence of liquid here means that the seal between the tip and the valve assembly has failed.

Remedies include replacing either or both parts, or trying to reassemble them with lower or higher tightening torque. Finger tight is all that is needed.

- be sure to lock the gradient into the holder by twisting the cap after inserting the tube all the way in.
- the keypad is made of lexan (polycarbonate) and is very sensitive to organic solvents including methanol. Clean with soap and water only.
- when using the new silicone tips, the larger SW28 PVC seals or very viscous solutions, there may be a 2-3 sec delay before drops stop emerging from the collection tubing after the piston has stopped. Pressure inside the tube during fractionation flexes the piston seal so it needs time to revert to its original shape. Be patient. If need be, program a Delay of 3-5 sec in the programmed rinse before air or rinse is introduced. Slower fractionation speeds reduce this problem.
- if the software freezes, simply reboot the machine and withdraw the piston.
- avoid cross-threading the tips when screwing them onto the piston. Always screw backwards until you feel the threads slip by each other, and then screw forward (clockwise)
- To erase all the stored gradients and rinses, go to the **SETUP** menu and press **DFLT** and **YES. All the gradients in memory will be erased and the 6 initial rinses restored to default values.**

SECTION 9. INTERNET PROGRAMMING INSTRUCTIONS

NOTE: The instrument is preprogrammed. This section describes how to install new software releases or reinstall the existing software from the SD Card supplied with the unit.

9.1 ENTERING NEW SYSTEM SOFTWARE.

Software updates are usually sent by email as .zip (zipped) files, and the SD card in the accessory kit is used to enter them into the Fractionator's flash memory.

1. Locate the SD card and its USB reader in the instrument kit. Insert the SD card reader in the USB port on your mac or PC and insert the SD card into the card reader label side up and gold fingers forward. Open the card on the desktop. Here is a list of the files as they should be labelled on the SD card:

   CFRCODEA.S19 This is the main software version (i.e. 7.70).
   SDFRprga.S19 This software loads the new software into the proper sector on the flash chip.

2. Drag and drop the new .zip files onto the SD card. **To avoid corrupting them, unzip them on the SD card**. Close the window and remove the SD card from the reader.

3. Insert the card into your instrument's SD card slot on the front panel

4. Turn the power OFF and then ON with the upper right most key pressed: hold it down until you see "OK", then let go.

5. Press the "FRLD" key, then "CODE".

6. When the display says RESET or SWXX enabled, turn the Station off and back on and you are ready to go.

Here is how he display looks:

```
BioComp Flash SDLder 1.5
LOAD MDSP      FRLD
```

Press the FRLD key. The display should show:

```
GRADIENT STATION SD v1.5
CODE
```

To load new main software, press the CODE key. The display shows

```
Downloading SD HEX file.
Rec loaded = 0 -> 2500
```

After a brief pause, the display starts counting up from 0 again:

```
Downloading SD CODE file.
Rec loaded = 0 -> 4400
```

The display then reads:

```
Downloading SD CODE file.
Wait,COPING Flash Page 7
```

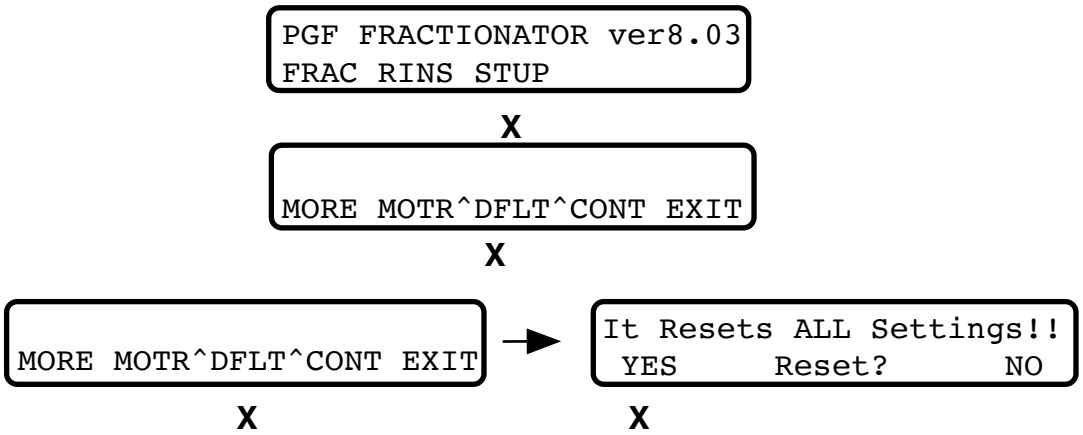
After ~40 sec, the display reads:

```
FLASH Programmed, Reset
Wait,COPING Flash Page 7
```

The main code is now loaded and you should turn the PGF off and back on to see if the program loaded properly. The display will show the current program version:

```
PGF FRACTIONATOR ver8.03
FRAC RINS STUP
```

If the display asks you to reset, say yes. Then go to the SETUP menu and RESET there as well.



SECTION 10. TROUBLESHOOTING.

10.1 Startup after installation: the motor doesn't run:

Symptom: The display lights up and the keypad and dial work, but the motor does not work in either direction

Solution: Check to see that you plugged the white motor plug into the circuit board as described in section 5.4. If the plug is misaligned the motor will run roughly or not at all.

10.2 The piston seal leaks during fractionation:

Symptom: As the piston descends through the gradient, liquid rises up around the seal.

First a clarification. The piston seal normally has a 0-4 mm "wet" zone at its bottom edge. You have a problem when this zone is larger than 4 mm high and gets considerably larger as the piston goes down. The inside diameter of the tube is slightly narrower at the bottom so a slight increase in the height of the liquid from increased compression is normal - as long as it never goes above 4 mm.

There are 7 possible causes of piston leaks:

1) The tip seal is compressed: When you run the tip all the way down to the lower limit switch, the seal is slightly compressed in the round end of the tube and may not expand back to its original size for the next sample. The solution is to re-expand the seal between runs by flaring it from underneath with your gloved finger. It is always best to go no further than 1.5 mm into the round end below the seam in the tube.

How to tell this? To check the depth of penetration, run the piston down until bottom edge of the seal is at the bottom seam in the tube where the wall joins the round end (mark this seam with a marker to make it essayer to see). Hit RESET and run it down to the limit switch. The distance should read 1.50 mm

Solution: After every run, reflare the seal by pressing it open with your finger.

2) The tip seal is old or damaged: remove the tip, dry it and examine the soft plastic seal under magnification to make sure there are no nicks or debris in the very bottom outer edge: it must be smooth. The seal will also shrink slightly with age and exposure to high heat.

Solution: If there is obvious damage, replace the seal. (see section 8.9 for a photo of a used seal)

3) The tube is defective. Examine the internal wall of the centrifuge tube. Because they are made by a process called blow molding, defects such as a deep groove that runs down the inside are seen occasionally. The groove is too deep to enable the seal to function properly and it leaks. Take a brand new tube and hold it up to a fluorescent light. Look down into the open end of the tube so the reflection of the light bounces off the INSIDE of the tube. You will notice that there is a seam in the bottom round part of the tube and that the seam carries up opposite sides of the tube. You may also see in the reflection that one of the seams on the wall of the tube has a slight (hopefully) or serious (bad) groove associated with it. If the groove is too deep, the seal can't reach into it and make a seal so the tip leaks as it goes down. It is easy to see if this is a problem. Fill a suspect tube with water and put the tube holder cap on it so the notch in the cap lies above the suspect seam - this will place it front and center when you begin to fractionate. Turn on the frac light and lower the piston into the tube. If the seam is leaking, water will shoot up right at the seam and nowhere else.

Solution: Replace the tubes after checking them for similar grooves. We will replace any defective tubes you find at no charge. Please alert us as soon as possible, giving the lot number on the box of tubes.

4) **The tube's wall is too thick.** The inner diameter of these tubes is not strictly controlled (see 3. above), so there might be an occasional tube with a thicker than wanted wall that is exposed in the cap's funnel and grabs the edge of the seal as it enters the tube. The result it that the seal's fine bottom edge catches on the sharp plastic tube and is bent backwards, leaking all the while. Tpo check a suspect tube, place it in the cap and take a sharp pointed object like a dissecting needle and slide it down the wall of the cap funnel and into the tube. If the tube wall is too thick, the needle will catch on it. The needle should slide straight down the funnel and fall off a ledge as it enters the tube, never grabbing the tube's edge. You can see the damage this does to the seal when you examine it under some magnification. Compare your damaged tips with an undamaged one so see if this shows up.

Solution: Discard the thick walled tube.

5) The tube has expanded during centrifugation and the seal leaks because it is too loose. This problem appeared with the SW60 (11 mm) seal when used with Dupont (Sorvall) centrifuges. The rotor has a tube bucket with a larger internal diameter than the Beckman so the tube expanded to fit it during centrifugation and then leaked during fractionation. We made the seal larger and it no longer leaks. If you have a similar problem, call us.

6) There is a leak between the seal and the hard part of the piston tip. Sometimes you can't see behind the tip and the drops are coming out between the two plastic parts.

Solution: Rotate the tube in the cap and the seal on the tip to see if you can bring the leak to the front where you can observe it during the run. If the leak persists, replace the tip/seal with a spare in the kit.

7) The piston is misaligned. If the piston is misaligned, it forces the tip to one side of the tube and breaks the seal.

Solution: Refer to the alignment section (7.12)

10.3 The tip will not tighten properly. If the tip spins without tightening, you have damaged the small tab the locks the valve in the piston (see section 8.2.7).

Cause: you have overtightened the tip onto the valve. It needs to be tightened gently to avoid this.
Solution: replace the valve.

10.4 The valve is not held into the piston.

Cause: the piston nut that holds the valve into the piston has broken. See section 8.2.3 & .7. The plastic nut has a thin wall that is fragile. Do not overtighten either the nut while installing the valve or the tips **.**

Solution: purchase a new nut

10.5 The Rinse and/or Sample flow is blocked.

Symptom: the volume of rinse slows to a trickle.

Cause: Dirty Rinse or gradient solutions clog the small orifices in the valve. Always filter your gradient solutions and rinses.

Solution: First, try the simplest fix: remove the red tower cover and take the rinse tubing off its needle. Connect a syringe full of filtered water to the end of the **Sample** line using the 20 ga cleaning needle and force water **backwards through the Sample lin**e and out the Rinse line. This usually is all that is needed.

If that fails, unscrew the piston tip from the valve and then remove the valve from the piston by unscrewing the nut that holds it to the piston. Disconnect the Air line from its needle and pull the valve out of the piston.

Now, carefully remove the bottom valve cap, the Oring and the ball as described in Section 8.3. Force filtered water down all three lines: Sample, Air and Rinse. If this fails, try HOT, SOAPY water.

Reassemble the cap with its Oring and ball as described in Section 8.3 and force water down the AIR line. It should come out both the Sample and Rinse lines with equal flow, while not coming out the bottom cap. If this fails, return the valve to us for repair.

10.6 The piston stalls on the UP stroke (especially near the bottom of the stroke).

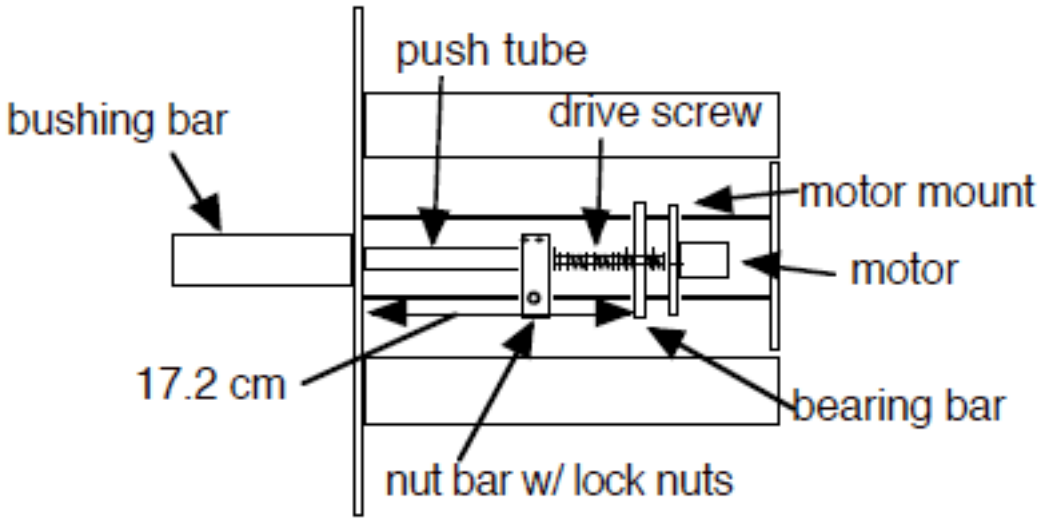
Symptom: You press the UP key and turn the dial and the piston starts to go up and then stalls. If you press STOP and press UP again, the same thing happens. The piston may not stall from the middle of its stroke to the top of its stroke.

Cause A: the unit has not been used for some time and the push tube has lost its lubrication.

Solution: The piston needs to be run UP and DOWN a few times to relubricate the Orings inside the black bushing tube. This can be done at a lower speed where the motor has more torque. Go the the SETUP menu in the MAIN MENU and press MORE to get to the SPED adjustment. Set the speed to 4.0 mm/sec. or less. EXIT to the MAIN MENU. If the piston is in the DOWN position, Press FRAC, then UP and turn the dial fully counter clockwise to start the piston UP. If the piston still stalls, set the maximum speed to 3.0 and try again. Run the piston up and down a few times to lubricate the Orings and return the SPEED to 6.0 - 6.5 mm/sec and see if that was the problem.

Cause B: the motor drive is misaligned and binding.

Solution: Remove the tube holder and run the piston to its fully DOWN position. Remove the computer panel and the upper front panel. Lay the unit on its back. Measure the distance between the top of the bearing bar and the bottom of the top plate. It should be 17.2 cm, but more importantly, it should be exactly the same on both sides. To adjust the distance, loosen the cap screws on the bearing bar and the set screws on the motor mount using the allen wrenches provided in the kit. Slide the two plates together until the distance is 17.2 cm on **both** sides of the bearing bar as shown below. Retighten one bearing bar screw, then the other, checking the distance often. When both sides of the bearing bar are aligned properly and tightened, tighten the two set screws on the motor mount.



Cause C: the set screw on the nut bar is too tight. The nut bar serves to prevent the push tube from rotating. It slides up and down on the 1/2" chrome tube, using two nylon-tipped set screws as guides. Each set screw is held in position with a lock nut. There should be a small amount of

"play" (looseness) in nut bar. If the set screws are over tightened, they will bind on the chrome rod and retard piston movement. The set screw is adjusted by loosening the lock nut, turning the set screw back and then in until it makes contact with the rod and then back out 1/5 turn. Hold the set screw in position with an allen wrench while you tighten the lock nut. Check the play by moving the nut bar front-to-back rapidly, listening for a tiny ticking sound as the set screws hit the shaft. The movement and the sound should be barely perceptible. If the screws are too tight, there is no sound and no movement as you push and pull on the nut bar.

Cause D. You have a poor connection between the circuit board and the motor. Unplug the motor plugs and the 17-pin plug and replug them.

10.7 Air pump stays on continuously.

There are **two** possible causes.

One is that you are using a rinse that has AC (air continuous) in the series. Go to the RINS menu and delete this part of the rinse or use a rinse that lacks AC.

The second is a short in the air pump wire that is letting the current go to ground through the pump. Normally, the ground wire is OFF and only turns on when you press the AIR key or the rinse calls for air. If the insulation on this wire is penetrated and is touching metal, the current can travel to ground through the short.

There are two likely spots for the short: in the hole at the bottom of the push tube where the wire heads up to the tower, and inside the tower where the wire crosses over the sharp end of the air pump bracket. Call us if the second cause seems to be the likely one and we will take you through the repair.

Good Gradients!

David Coombs
dhc@biocompinstruments.com

You will be able to run your gradients, no matter what. The worst case is that you remove the tower cover, pull the rinse line off its needle, connect a syringe to the tubing using the cleaning needle and manually rinse the tubing.

REPAIR:

1. If you have a leak

1. Remove the square, flat top front panel so you can see inside. The only dangerous voltage inside is in the very back left corner of older Stations and Fractionators where 100-240VAC power enters, so stay away from there. Everything else is low voltage DC (12-24V). If your unit has an **external** 12VDC power supply, there is no dangerous voltage inside. In the newer 12VDC units, cover the caged power supply inside with multiple layers of towels to keep it from getting wet.

2. Before you rinse, look at the tubing's path for obvious breaks or disconnects. Pull all the tubing joints into and around the pump to see if they are firmly attached.

3. Fill the reservoir half full with water and, with the purge toggle switch down and a small beaker under the curved tubing below the toggle, very briefly press RINSE to prime the pump . If the water doesn't blast into the beaker, the pump is faulty.

4. Flip the toggle up and very briefly press the RINSE button, watching the tubing and pump for signs of an internal leak.

5. If there is a leak in the bronze fittings, make sure they are tight and retry.

6. If the tubing is leaking at the pump, force it onto the pump's nipple. If this is too difficult, remove all the water from the reservoir, pump and tubing using a syringe and warm the end of the tubing with a heat gun. When the tubing is very warm, it should be able to be forced onto the pump's nipple (always wet the nipple before applying any tubing).

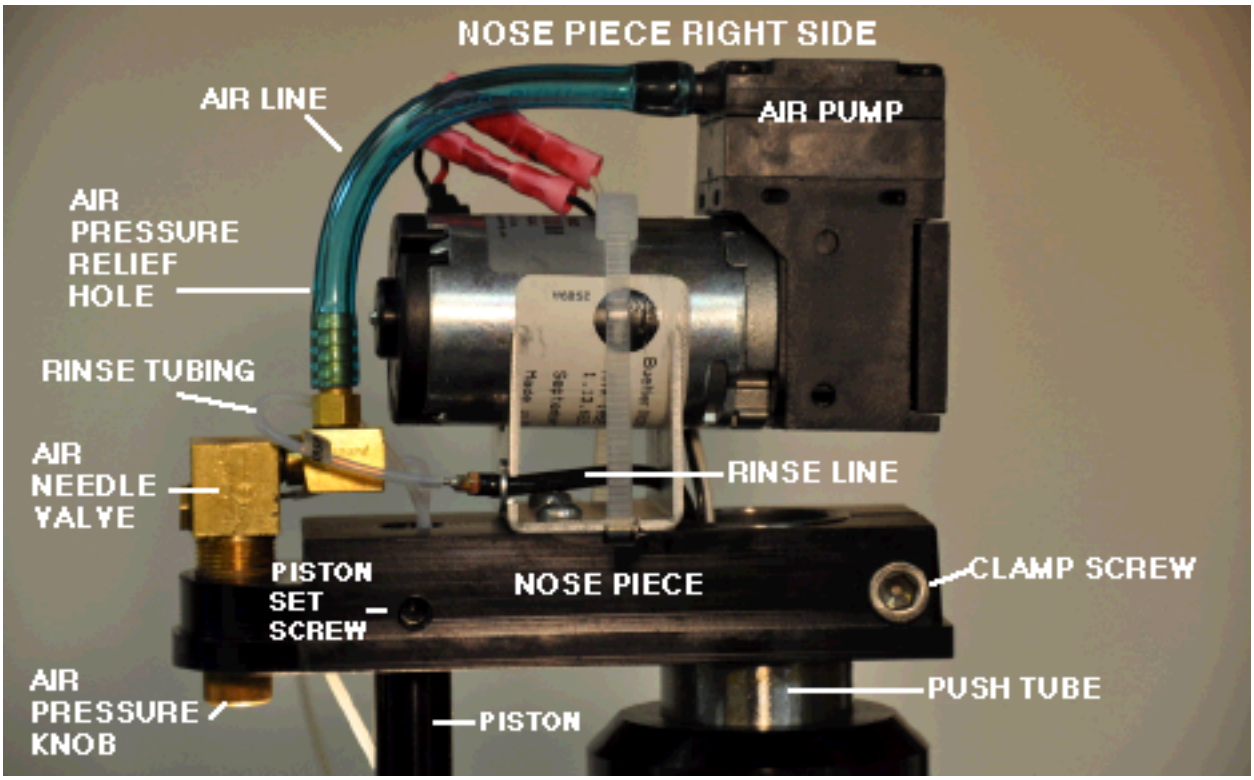
7. If the leak is where the black rinse tubing that carries rinse up to the piston meets the brass quick connect, push the tubing down into the quick connect to seat it.

2. Locate a blockage in the rinse line.

1. First, if you have a UV monitor connected, take it out of the line by removing the sample tubing coming from the piston to the flow cell. Try pressing the rinse button. If you get a solid flow out the end of the sample tubing, the flow cell is clogged. Remove the EM-1 gun from the side of the tower and then remove the flow cell itself by removing the metal clamp that holds the Luer ends in place and then loosening the small thumb screw near the IN/OUT end of the gun. Use a syringe filled with .2M NaOH and 1% SDS connected to the flow cell's top **OUT** port to see if that cleans it out. Immediately rinse with water to remove the caustic rinse.

2. If the rinse flow is weak with the flow cell disconnected, the block is on the rinse system itself. Remove the top tower cover to expose the air pump. You will find the black rinse

tubing ending under it, with a needle connecting it to the teflon rinse tubing that goes down the piston. See the photo below.



3. Remove the teflon tubing from the needle and press the rinse button. Rinse should shoot out across the room (hold a beaker in front of the needle to catch the rinse).

4. If that goes OK, your valve is clogged by particles in your rinse. Swap out the clogged valve for the spare in the kit. You should always filter your rinse water before use.

Please read the valve chapter in the manual starting on page 37 to see how to diagnose and fix your valve problems.
3-row frac manual v7.5 https://www.dropbox.com/sh/d6ytbroqi188g9b/AJXQ97adnz

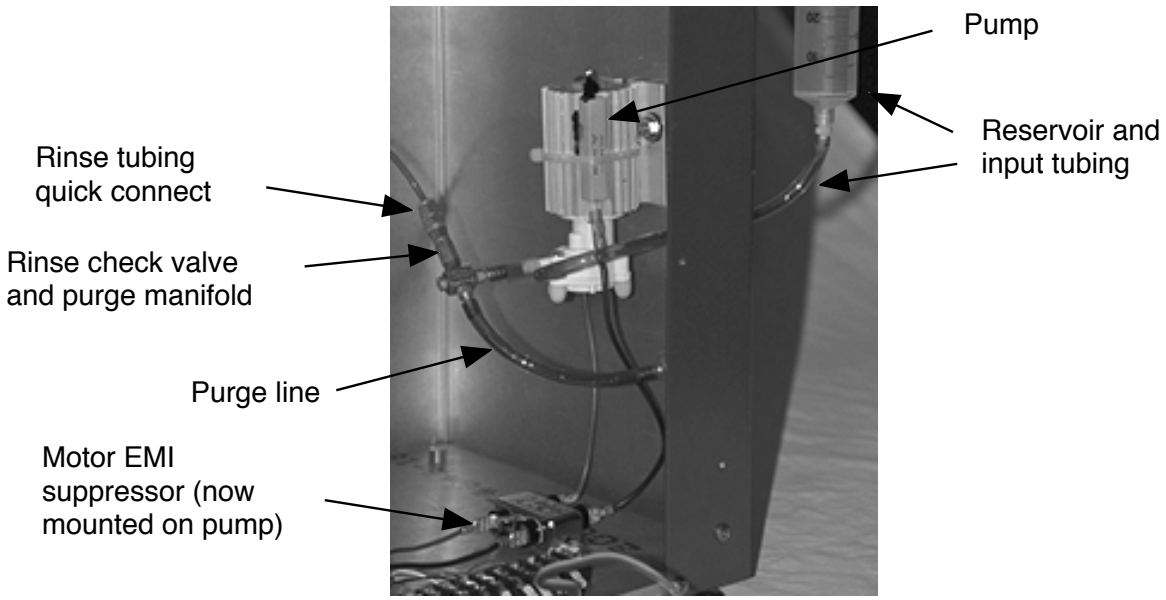
5. If water does not shoot across the lab in 3., then the clog is probably in the nipple itself. Push the teflon rinse line off the needle and remove the black rinse tubing from the notch

in the air pump bracket. Push the small washer back down the tubing a bit and grab the brass nipple with a pair of pliers. Pulling the nipple straight out of the tubing is futile, as this only tightens the grip. Hold the nipple at right angles to the tubing and you will break the seal and is should be much easier to remove.

6. Use a water-filled syringe and the 20 ga cleaning needle in the kit to force liquid backwards into the needle to remove any clog. If needed, use a 24 ga needle to unclog it. When you force water through the disconnected nipple, you should see a thin smooth stream of water.To make sure the rest of the rinse system is OK, hold a beaker in front of the black tubing and press the rinse button.

7. To make sure the rest of the rinse system is OK, hold a beaker in front of the black tubing and press the rinse button. Water should gush out the black tubing.

8. If 7. gives weak flow. look inside the box, you will see the black rinse tubing emerging from the push tube and going to the pump. The black rinse tubing ends in a quick connect fitting. To remove the tubing, push the brass ring on the fitting down while pulling the tubing out of the fitting. Push down hard on the ring while pulling the tubing out with force.



Hold a beaker in front of the fitting and press the rinse button again. Still weak? You have a clogged check valve or a bad pump.

9. To check the check valve, use a pair of pliers and remove the check valve. It has a half cylindrical/half hex body and unscrews from the 4-hole manifold piece at the "T" junction of the tubing. Repeat the rinse with beaker in hand. Still weak? Pump needs to be replaced.

10. Reassemble the Rinse system in reverse.

Report any other problems directly to me.

PREVENTION:

1. clean the reservoir and label it RESERVOIR in big letters. Never insert a plunger into it from this time onwards.
2. Label a fresh 60 cc syringe AIR and only use it to dry out the tubing at the end of the day. NEVER remove its plunger and discard it when the lubrication inside is lost.