



MODEL 153

GRADIENT STATION

Manual version 3.3

SD Card Memory System

Fractionator software version 8.03

Gradient forming software version 5.3

OPERATOR'S MANUAL

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May 22, 2013

GRADIENT STATION
BIOCOMP Model 153
Gradient forming software Version 5.3
Fractionation 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 GRADIENT STATION, 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 instrument 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 instrument 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 Gradient Station combines the best of BioComp's two premier gradient instruments in one. The Gradient Master is embodied by a tilting and rotating disk on the right wing of the Piston Gradient Fractionator.

Gradient forming is accomplished the the patented "tilted tube rotation" method invented by Dr. David Coombs, the founder of BioComp. This remarkably simple and reproducible system involves layering the 2 end point solutions in the tube, capping the tube and rotating it for short time at a predetermined angle. The rotation of the wall of the tube smears the interface between the light and heavy solutions, forming the desired gradient.

The Piston Gradient Fractionator (PGF) was also invented by Dr. Coombs (Coombs, 1974, Anal. Biochem. 68:95-1011) and offers a new dimension in fractionation. A piston is forced into the centrifuge tube from above and it seals against the inside wall of the tube, forcing the gradient out of the tube layer by layer. 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 converts 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 standard needle puncture devices (see the web page for the actual data).

Dr. Coombs' work with the purification of viruses led him to develop an exquisitely sensitive band visualization system. T4 phage at $>10^8$ phage/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. In the future, we will provide UV sensitivity 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 system 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 you turn the dial, 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 **first drop** to leave the tubing. This will become the "TOP" of every gradient, and fractionation can proceed accurately below this point if you press the RESET key to zero the device at this position. The rest is described below.

SECTION 2. SPECIFICATIONS

2.1 Physical Measurements

Shipping Weight: 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: 90-240VAC, 0.4A, 50-60Hz.

2.2 Commercially available parts:

Stepper Motors: All Hurst (Princeton, Indiana) Fractionator; TS -20 reduction cat no. SP3885; Tilt PBS - 150 reduction, 3208-002; Rotate PBS - 20 reduction 3208-009.

Power Supply: 12VDC, 5A 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:

Gradient Forming:

Tilt Angle: 0-90° in 0.1° increments.

Rotate speed: 0-60RPM

Fractionation:

Piston Speed: 0.1-6.5 mm/sec down, 6.5 mm/sec upward

Fraction Size: 0.01 mm - 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):

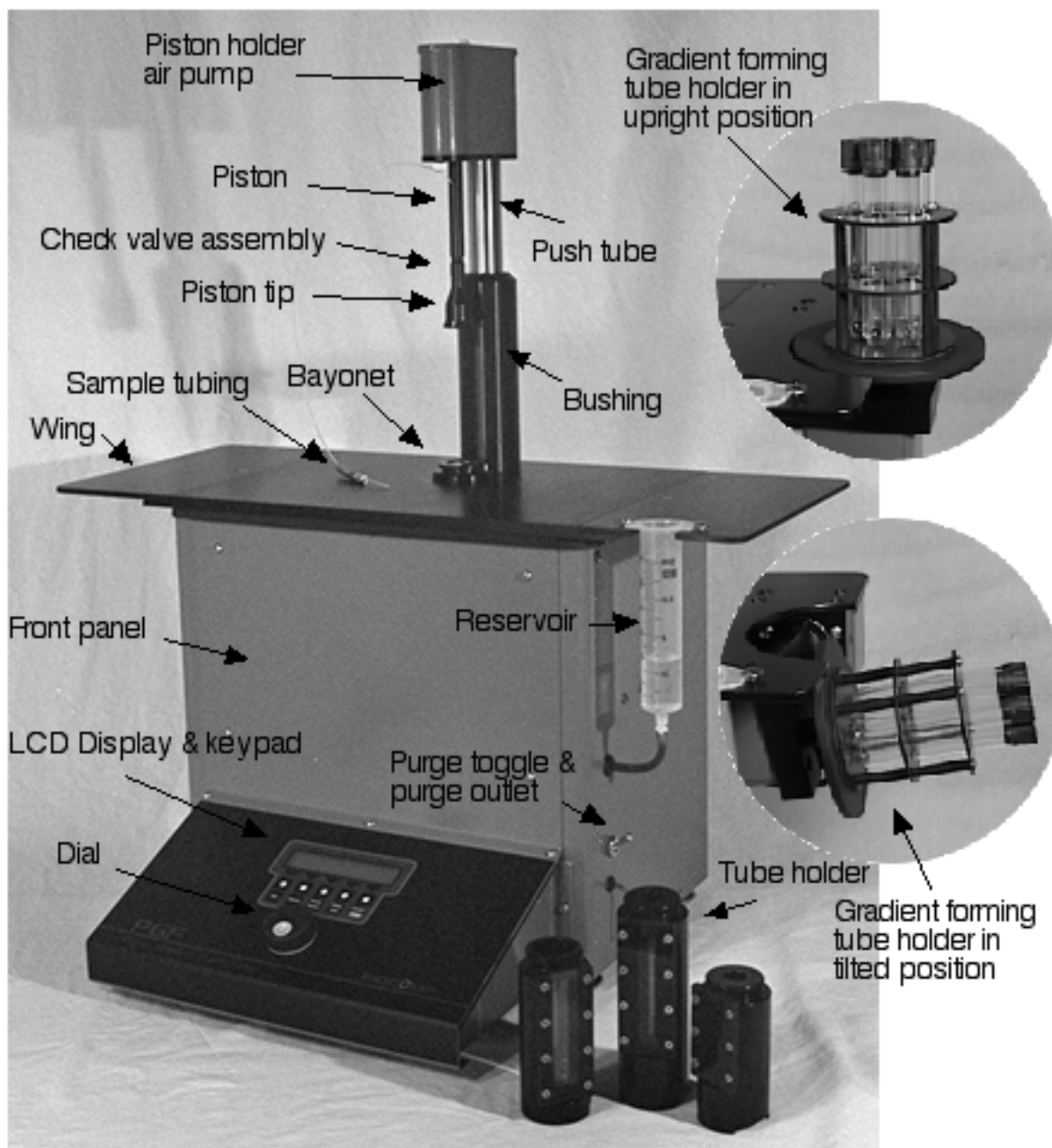
- Continuous air to section outflow with air bubbles during fractionation
- Air on demand or automatically between fractions: 15 msec to 9 sec
- Water/Buffer on demand or automatically: 15 msec to 9 sec
- Delay (a programmed interval between fractions): 15 msec to 9 sec
- Pause (an indefinite pause between 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.

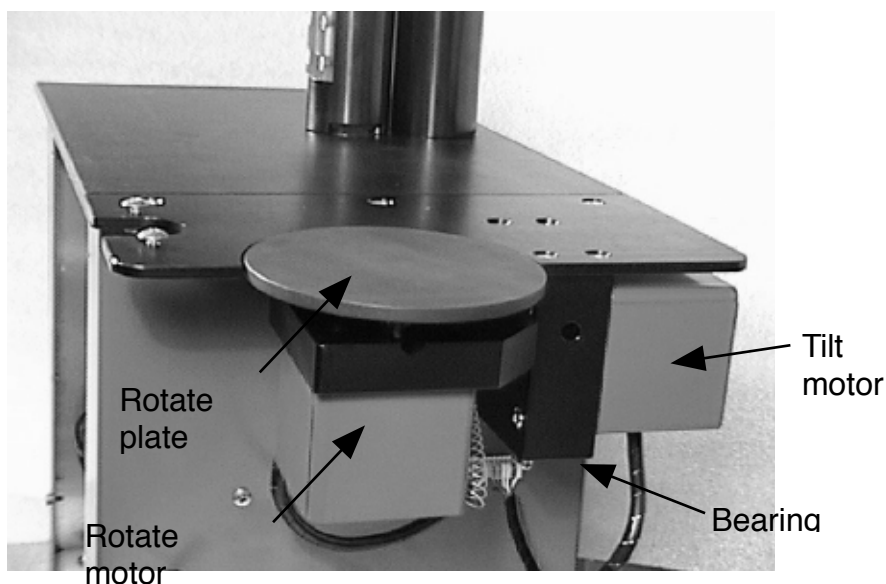
Macro Record: The device will reproduce a manual run if it is recorded using this feature. The memory becomes one of the 20 memories listed above and can be edited and saved.

SECTION 3. PARTS DIAGRAMS

3.1 THE GRADIENT STATION



3.2 GRADIENT FORMING ATTACHMENT



SECTION 4. ORDERING INFORMATION

The Gradient Station™ gradient forming and fractionation system.

4.1 **Base Unit:** Cat No. 153-001 90-120VAC input;
153-002 220-240VAC input

4.2 Gradient Forming Accessories

2 cannula

1 bubble level

4.2.1 **Tube Holders:** (each tube holder is shipped with a marker block and 6 or 12 caps, I, R or IR)

105-925-I, R or IR: 25 mm SW28 tube holder

105-916-I, R or IR: 25 mm SW28.1 tube holder

105-914A-I, R or IR: 25 mm SW41 tube holder

105-914B-I, R or IR: 25 mm SW40 tube holder

105-913-I, R or IR: 25 mm SW50 tube holder

105-911A-I, R or IR: 25 mm SW60 tube holder

105-911B-I, R or IR: 25 mm TLS55 tube holder

(8-place tube holders are available for quick seal vertical rotor tubes, but they cannot be fractionated with this device)

4.3 Gradient Fractionation

Includes the following accessories:

1 Manual

1 60 cc syringe rinse reservoir

4 feet

1 Set of 6 Allen Wrenches,

8 Spare check balls and 2 sizes of orings

1 Cursor for each tube holder

1 Spare light bulb

1 Spare valve assembly

1 valve cleaning needle

1 m of spare Teflon tubing

1 fine marker

1 fine marker

1 Left wing for extended working area

1 Front panel keypad / LCD display / computer

1 Fraction collector advance cable

1 Phillips screw driver

1 Tubing terminator

2 sets of assembly screws for computer panel and wings

1 Water level adjust cannula w/ stop

1 power cord

1 12VDC 6.6A transformer

1 SD card with pdf manual and backup files

1 SD card reader with USB cable

1 SD LEXAR card reader with USB cable

- 4.3.1 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

4.3.2 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

4.3.3 Spares:

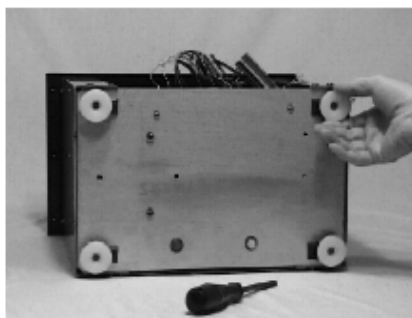
- 151-2XX-4 (XX is the mm diameter of the tube) 4 Piston tips with seals
- 151-902 12V 20W Spot light with dichroic reflector
- 151-905 check valve assembly for piston air/rinse/sample
- 151-905-11 check valve assembly for piston air/rinse/sample for 11 mm tip
- 151-931 Bag of 50 mist filter discs
- 151-919 2 each 1/16" ø stainless ball 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 BIOCAMP 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.

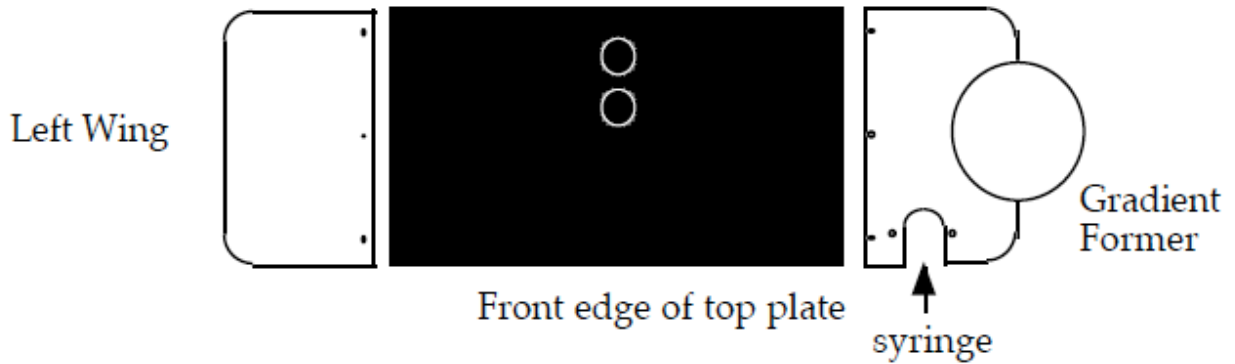
Locate the 4 feet in the accessory pack and screw them into the holes left by the bolts. Level the instrument on the bench where it will be used by screwing the feet in or out as needed. Be sure that all the parts listed on the shipping papers are present.



SAVE THE SHIPPING BOX FOR POSSIBLE RETURN OF THE INSTRUMENT.

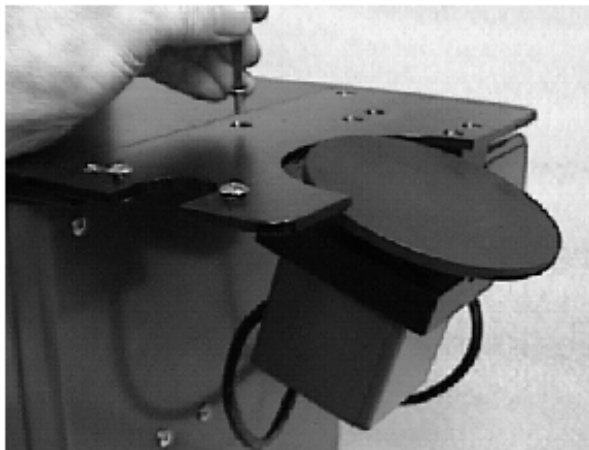
5.2 Assemble the Left Wing:

There is a D-shaped black plate in the box. Use the flat head screws and the 1/8" hex wrench to assemble it to the top plate in the position shown. It extends the working area for tube racks.

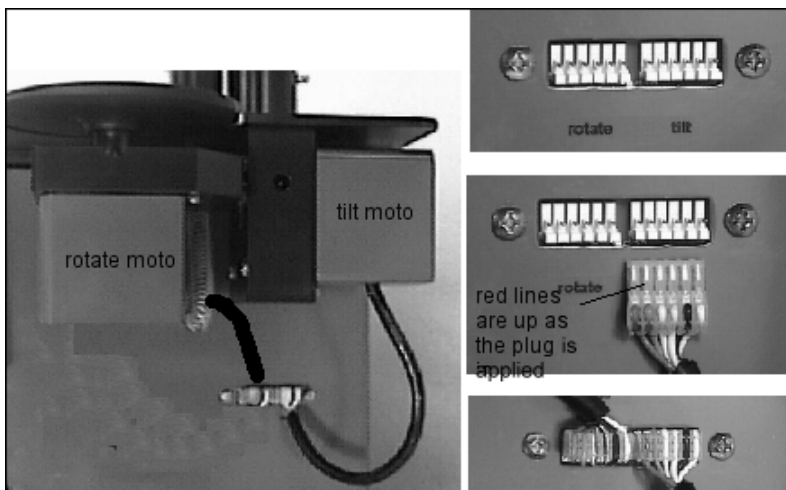


5.3 Assemble the Gradient Forming attachment:

Carefully remove the Gradient Forming attachment from its box and attach it to the right wing position. While supporting it, screw in one of the same flat head screws you used for the left wing, then apply all three screws.

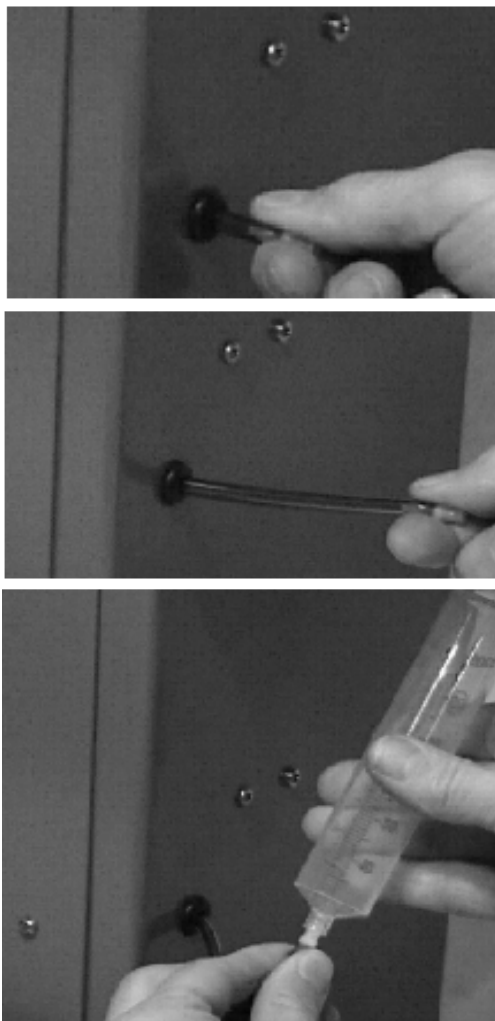


When the wing is secure, apply the two motor plugs to the sockets on the right side of the instrument as shown. Be sure that the plugs are all the way on, touching the white plastic on the socket.



5.4 Attach the Reservoir:

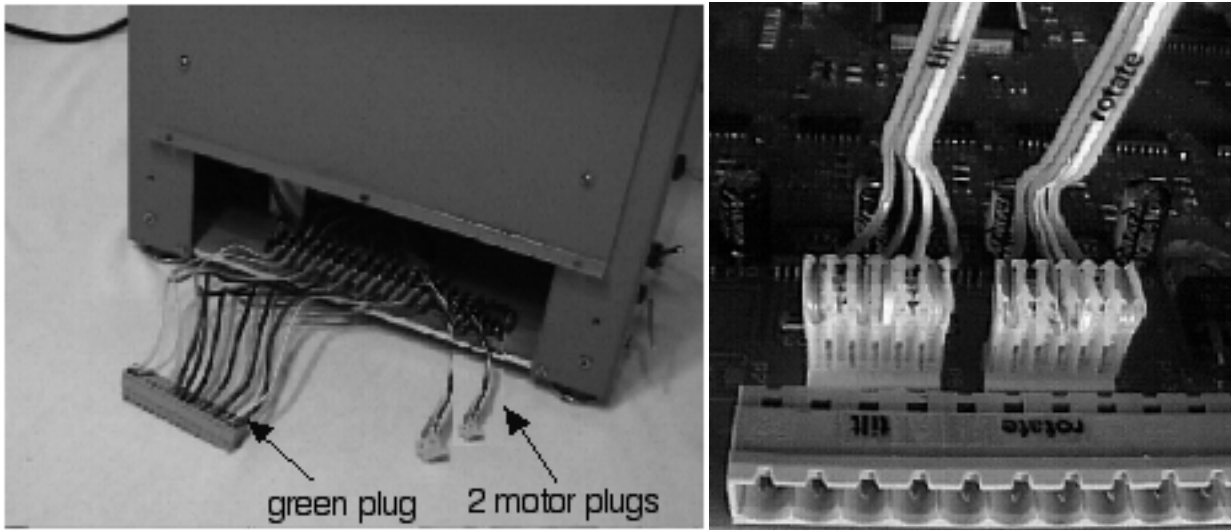
There is a 60 cc syringe in the accessory kit. Pull the plunger out and connect it to the luer-loc connector at the end of the blue tubing on the right side of the box (pull the tubing out first). Slide the flanges on the top of the syringe barrel under the screw heads on either side of the notch in the right wing. Fill the reservoir with **FILTERED** water or buffer, depending on your needs. Small particles of dust and debris can clog the valve in the piston tip. The salt in buffer will reduce the carryover between fractions and keep your samples isotonic. However, it will also put more load on your scintillation cocktail if you are counting your rinses.



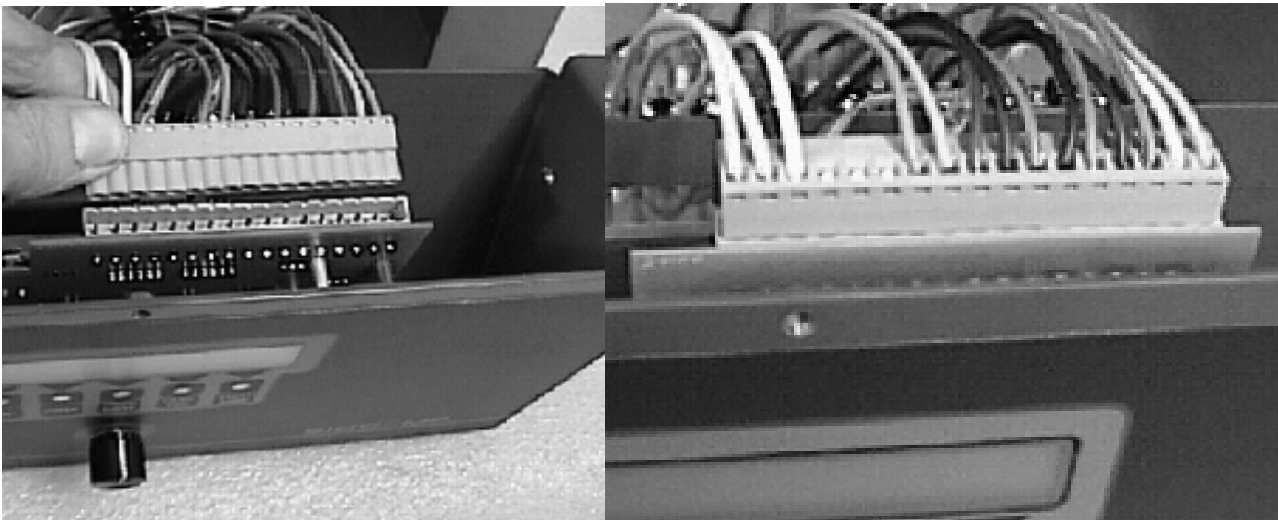
5.5 Assemble the computer panel.

Find the green multi-wire plug and the 2 smaller, white 4-wire motor plugs inside the main unit and expose them as shown below. Note that the wires on the plugs are labelled "tilt" or "rotate".

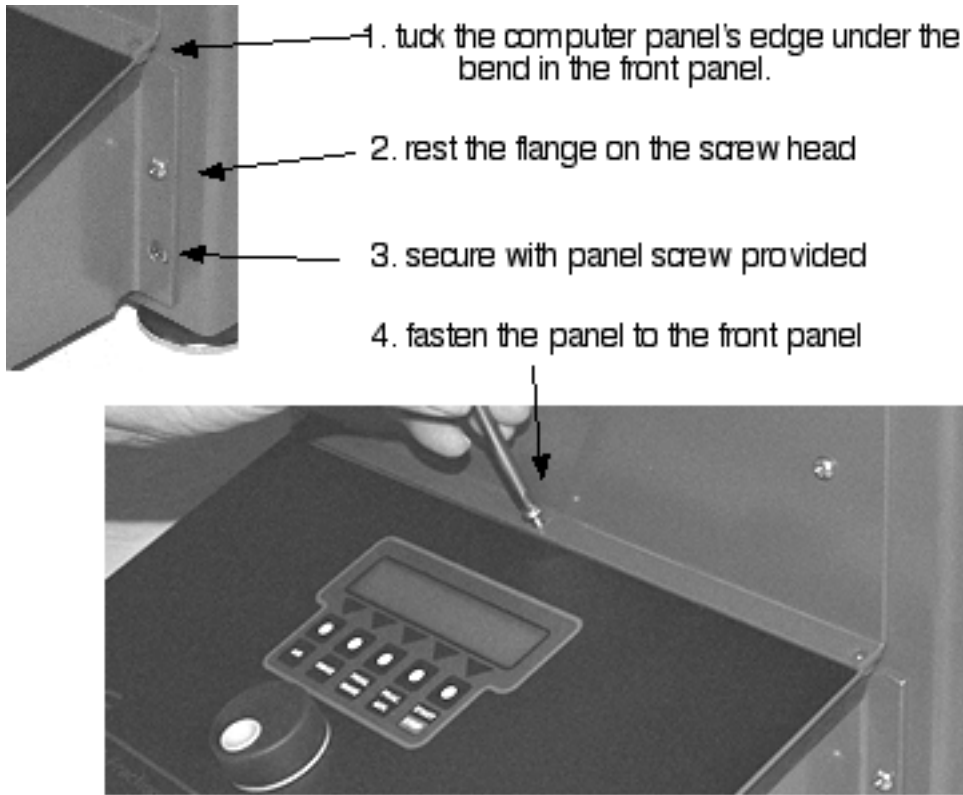
Bring the front panel close to the instrument with its open end up. Observe a green socket along the front edge of the circuit board and two 6-pin sockets labelled "tilt" and "rotate". Insert the motor plugs onto their 6-pin sockets. Note that the motor plugs have red stripes on one side. They must face 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 green socket on the back edge of the circuit board. **Push it all the way in very hard.** The plug can only be inserted with the scalloped edge up so there is no danger of inserting it incorrectly.



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 the two flanges on the screw heads already installed on the side panels of the main unit as shown. Next screw the computer to the bent edge of the front panel using 3 more 8-32 screws as shown:



5.6 Install the power plug.

You are now ready to power up the unit.

Find the external power supply (12VDC Powergate transformer) and insert its plug into the socket on the right rear corner of the instrument. Non-Euro and North American users must supply the power cord between the transformer and the wall socket. 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.



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

GRADIENT STATION	v1.03
FRAC	GMST

X



The instrument is preprogrammed and is now ready to use

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. When an X appears beneath a word, it means that its smart key has been pressed.

The dedicated keys in the second row are used only for fractionation and 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. GRADIENT FORMING - STATION

1.1 LEVELLING THE PLATE: A CRITICAL FIRST STEP

1. When you power up the device, the display reads:

```
GRADIENT STATION  v1.03
FRAC              GMST
                  X
```

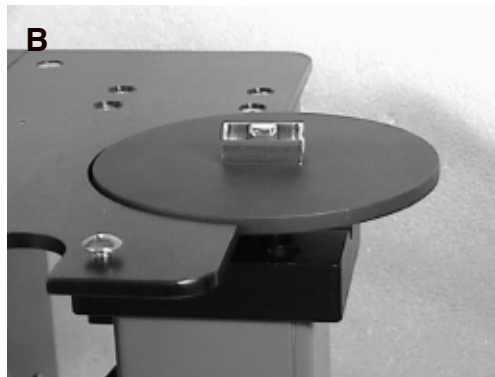
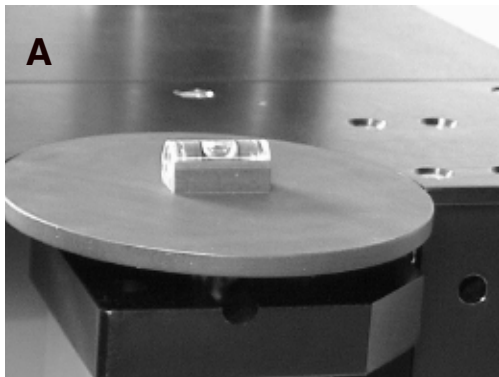
Press GMST. After the Gradient Master software has loaded, the display will read:

```
Reset KNOB to 0 position
Position = 29
```

Turning the knob until the indicator line is up center (12 o'clock) changes the display to read:

```
LEVELLING, TURN KNOB
DONE
```

Find the bubble level in the accessory kit and place it on the center of the plate. Level the unit in two stages. First, place the level so it runs front-to-back as shown in (A). Adjust the feet under the corners of the instrument until the level is centered. Next, turn the level so it runs side-to-side as shown (B).



Turn the knob clockwise or counter clockwise to level the plate. For slight adjustments, use slight turns of the knob; for course adjustments, turn further. There is no harm to the motor if the round plate contacts the top plate. When the round plate is level, press DONE. This is an essential step in forming gradients because the device uses the levelled plate (0°) to calculate all the tilt angles.

2. Levelling the plate during use. Press the EXIT key until the display shows the Main Menu;

```
GRADIENT MASTER ver5.3
GRAD      SYST
          X
```

Refer to the Gradient Master Manual that follows for the complete directions on gradient forming: