Affymetrix® GeneChip® Fluidics Station 450/250 User's Guide

P/N 08-0092 Rev. G
April, 2006

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Manufactured by

Affymetrix, Inc.

Bedford, MA 01730

Contents

Chapter 1

The Fluidics Station 450/250

Chapter 1

Introduction

This chapter introduces the Affymetrix® GeneChip® Fluidics Station 450/250 and its components, gives an overview of how the fluidics station works, and covers the safe use of the fluidics station.

Note that the Fluidics Station 250 is identical to the 450 except that, instead of four modules, only two modules are present.

The information in this user guide pertains to both models.

FS450 FEATURES

Table 1.1
FS-450 Features

Feature	Description	Benefit
3 position sampling with individual vial detection	• System will accept 1 to 3 vials. • System can detect the presence/absence of vials in the 3 loading positions.	• Flexible loading and operation to meet script and user requirements.
Unattended operation ("Walk away capability")	• System will automatically sample from up to 3 vials based on defined protocol.	• No interruptions to operator during the protocol – simply "load and go." • Labor savings.
Modular design	• Individual faceplates and blanks provide the possibility to offer fluidics stations with two or four modules.	• Fluidics processing capability can grow with GeneChip probe array usage. • The operator can replace a failed module.
New probe array cartridge loading	• Linear (versus angular) movement of the cartridge to engage fluidic connections. • Features on the probe array housing used to align fluid needles with cartridge.	• Easier cartridge loading. • Secure fluidic connection. • Reduced potential for leaks.
New fluid detection sensor	• Presence/absence of fluid detected by measuring resistance across the needles that penetrate the GeneChip cartridge septa. • Detection path automatically replaced by loading cartridge.	• Robust performance. • Elimination of false positive and false negative missing fluid errors caused by sample carryover.
Leak path isolation	• All potential leak paths are funneled into a single channel.	• Critical fluidic components are protected from potential damage. • Should leaks occur, single channel enables rapid identification and resolution by user.
Improved user diagnostics	• Introduction of new error codes to software.	• User can quickly identify/ correct faults on their own to get running without need for service call.

WARNINGS AND PRECAUTIONS

- The fluidics station is for research use only. It is not for use in diagnostic procedures.
- All biological specimens and materials with which the operator may come into contact should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations—including adherence to the OSHA Blood Borne Pathogens Standard (29 CFR 1910.1030) for blood-derived and other samples governed by this act. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
- Wear gloves when using the fluidics station.
- Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic reagents.
- Do not send your instrument elsewhere for service or attempt to service it yourself. To protect your warranty and ensure safe operation, the instrument should be serviced only by Affymetrix or its representatives. If the instrument is not working correctly, please contact your Affymetrix Technical Support representative.
- Do not use the fluidics station in ways not specified by Affymetrix. Doing so may impair the protections provided by the fluidics station.

WARNING ▲

Do not place hands or fingers inside the cartridge holder. Under fault conditions, the area behind the cartridge holder can have temperatures that rise to 100°C or higher.

- The fluidics station requires two people to lift and handle it safely. Each person should firmly grasp the base of the instrument at the end opposite the other to lift. Use OSHA standards for lifting techniques.
- The FS450 is intended for indoor, laboratory use in a controlled environment.

Caution Notices:

CAUTION

- You must have read and understood the contents of this manual before attempting to operate this fluidics station.

CAUTION

- The power supply cord is used as the main disconnect device. Ensure that the socket outlet is located and installed near the equipment and is easily accessible.

ATTENTION

- Le cordon d'alimentation est utilisé comme interrupteur général. La prise de courant doit être située ou installée à proximité du matériel et être facile d'accés.

ACHTUNG

- Zur sicheren Trennung des Gerätes vom Netz ist der Netzstecker zu ziehen. Vergewissern Sie sich, daß die Steckdose leicht zugänglich ist.

REGULATORY

This device complies with Part 15 of the FCC rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesirable operation.

Compliant with Directive 2002/96/EC (WEEE).

Compliant with EU Directive 89/336/EEC (EMC)

Compliant with EU Directive 73/23/EEC (LVD)

CUSTOMER SUPPORT INFORMATION

Affymetrix, Inc.

3380 Central Expressway
Santa Clara, CA 95051
USA

E-mail: support@affymetrix.com
Tel: 1-888-362-2447 (1-888-DNA-CHIP)
Fax: 1-408-731-5441

Affymetrix UK Ltd

Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH
United Kingdom

E-mail: supporteurope@affymetrix.com
Tel: +44 (0) 1628 552550
Fax: +44 (0) 1628 552585

Affymetrix Japan, K. K.

Mita NN Bldg
16 Floor, 4-1-23 Shiba,
Minato-ku, Tokyo 108-0014
Japan

Tel: (03) 5730-8200
Fax: (03) 5730-8201

www.affymetrix.com

Instrument Components

The GeneChip® Fluidics Station 450 contains four modules. The Fluidics Station 250 contains two modules. Except for this difference, the two fluidics station models are identical. Each module can hold one GeneChip® probe array cartridge and up to three 1.5mL vials. The Affymetrix® GeneChip® Operating Software on the computer workstation, which is connected to the instrument, can control each of the four modules independently of the others. A single computer workstation can control as many as eight fluidics stations. You can use any or all of the modules at the same time. You can also choose a different protocol for each module as long as each uses the same Wash A and Wash B reagents. The modules are numbered 1 through 4 near the LCD window.

The GeneChip Fluidics Station 450/250 includes the following components. See Figure 1.1 on page 11.

1. Sample Holders — holds up to three sample vials
2. Module Door — protective cover for the peristaltic pump on the module
3. Cartridge Holder — holds the cartridge during fluidics operation
4. Washblock — part of the cartridge holder that completes the fluid path when a cartridge is not in place (used for cleaning out or draining the fluidics station)
5. Cartridge Lever — engages or releases the cartridge holder
6. Needle Lever — inserts the needles into the sample vials
7. LCD Window — displays messages during processes
8. Wash Bottles (2) — hold wash buffers and tubing that draws buffer through system
9. DI Water Bottle — holds deionized water and tubing that draws water through system
10. Waste Bottle — collects waste from hybridizations and washes
11. Sample or Vial Needles — extend into the sample vials and draw fluid.

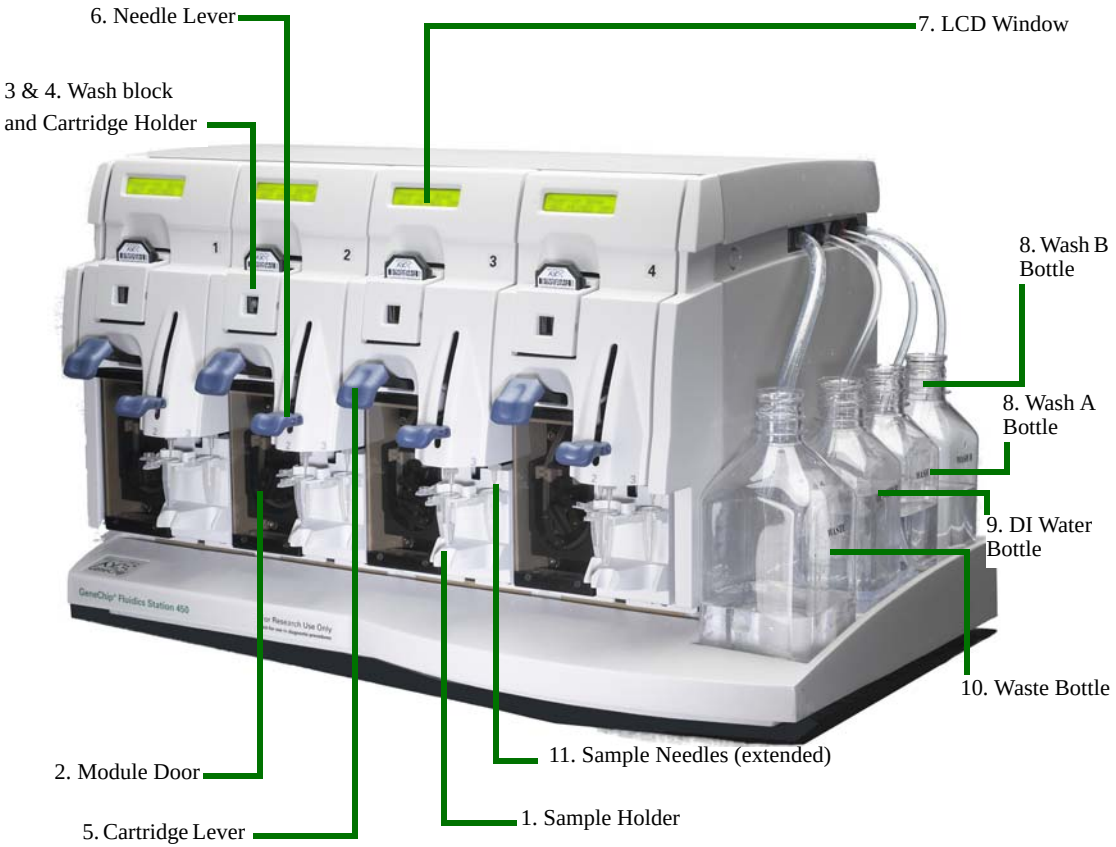

Figure 1.1
The GeneChip® Fluidics Station 450 and components

Accessory Items

Affymetrix provides the following accessories for Fluidics Station 450/250.

Table 1.2
FS-450 accessories list

Part Number	Description	Quantity
340011	Assy, Terminator Plug	1
350001	Assy, cable, CPU, DBP9,3/F	1
350014	Cord Power	1
370013	Fuse 4A, 5X20mm, Type F, 250V	2
400110	Tubing, Silicone Peristaltic, 8.5	4
08-0092	Fluidics 450-250 User's Guide	1
400118	Media Bottle, SQ, 500ml Fluidics DI water/buffer	3
400119	Media Bottle, SQ, 1000ml Bottle	1
400137	Bottle cap, Pre-drilled	4
410016	Needle, Sample	1
500321	Datum, Chip_49*	4
08-0093	FS450 Quick Reference Card	1
700357	MSDS Buffer, SSPE 20x	1
08-0099	Module Replacement Quick Reference Card	1
08-0076	Tough-Spot Quick Reference Card	1
610175	CD, Library files, Fluidics Station 450 Scripts	1

**Chip_49 is a blank probe array for testing purposes.*

How the Fluidics Station Works

For further instructions, see *Using the Fluidics Station 450/250* on page 15, and the *GeneChip® Expression Analysis Technical Manual*, P/N 900365 (available at www.affymetrix.com/support), for instructions on using the fluidics station with individual protocols.

SAMPLE STAINING PROTOCOL

1. Use the GCOS software to define an experiment and start the fluidics station protocol.
2. After the hybridization of the labeled target to the probe array, place the probe array cartridge into the cartridge holder on the selected module of the fluidics station. **Gently** flip the cartridge lever to close the washblock and engage the probe array.
3. Depending on the fluidics protocol for the array, place vials containing the proper reagent in individual vials in the vial holders as indicated on the fluidics station LCD. Please note that the indicated position may be different depending on the particular assay protocol used. Refer to the *GeneChip® Expression Analysis Technical Manual*, P/N 900365, or the applicable genotyping manual for details.
4. The fluidics station will perform the following actions to stain the bound target on the cartridge and prepare it for scanning. The fluidics station will:
    - wash the cartridge with wash solution (or solutions) at a selected temperature;
    - draw staining solution from the vial into the cartridge and mix it by alternately draining and filling the cartridge at a selected temperature;
    - expel the staining solution to the waste line;
    - fill the cartridge with wash solution for scanning;
    - clean the module tubing and needles for the next cartridge to be processed.
5. After washing and staining, the Affymetrix® GeneChip® Scanner 3000 scans the cartridge by laser light to obtain fluorescence intensity data.

For more information about how the scanner works, refer to the appropriate scanner manual.

In addition to the preceding protocol, the fluidics station can perform variations of the steps described in the protocol and can run other protocols. For more information about the protocols that can be performed on the fluidics station, please contact your Affymetrix® Technical Support representative.

Chapter 2

Using the Fluidics Station 450/250

Chapter 2

This section describes how to use the Affymetrix® GeneChip® Fluidics Station 450/250 with fluidics protocols.

Starting the Fluidics Station

1. Check to ensure that the fluidics station is connected to the power main through the power cord provided.
2. Check to ensure that the fluidics station is connected to the workstation. CommLink connections are located on the back of the fluidics station (Figure 2.1).
3. Flip the ON/OFF switch for the fluidics station to the **ON** position. The switch is located on the left side of the fluidics station (Figure 2.1). The LCD window should display the following:

Power-On Done **NOT PRIMED 25°C**

4. Turn on the computer workstation and launch the GCOS software.

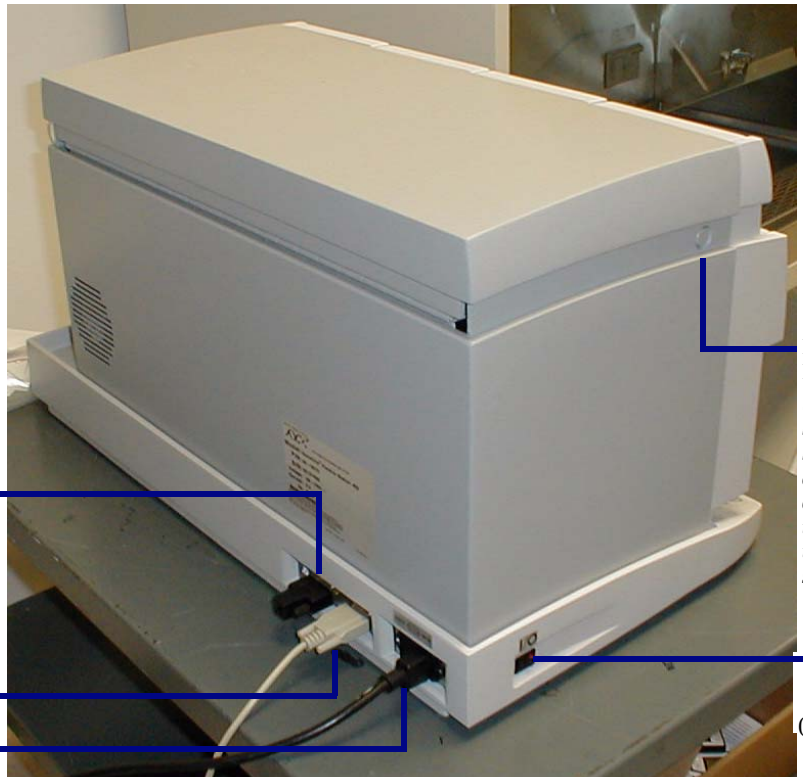

Figure 2.1
Location of the serial ports and ON/OFF switch

Handling the Probe Array Cartridge

The GeneChip® probe array chip comes mounted in a plastic package to form a cartridge (Figure 2.2). The probe array chip contains a collection of oligonucleotide probes that have been arrayed on the inner glass surface. A chamber in the plastic package directly under the chip acts as a reservoir where hybridization, washing and staining occur.

Although the inner glass surface is protected, any contamination or scratches on the outer surface of the glass can compromise the integrity of the scan. Avoid touching the surface of the chip with your fingers. Skin oils and other

substances, such as lotions or ink, can fluoresce. If the surface of the probe array chip is noticeably dirty, you should carefully clean the chip with a nonabrasive laboratory tissue.

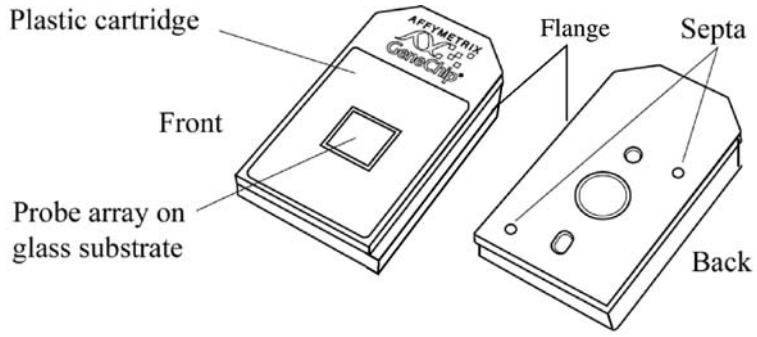

Figure 2.2
The GeneChip® probe array cartridge

Setting Up an Experiment

Before running a protocol on the fluidics station, you must first define an experiment in the GeneChip Operating Software (GCOS). For information on defining an experiment, refer to the GCOS software user's guide or to the appropriate package insert.

For more specific information on hybridizing the target to the probe array cartridge, refer to the appropriate package insert.

Defining the Experiment

In the GCOS software, open the **Experiment Info** dialog box under the **Run** menu. An **Experiment Information** dialog box appears.

1. In the **Experiment Information** dialog box, fill in the name of the experiment and select cartridge name from the drop-down list. Do this for each experiment. Refer to the GCOS software user's guide or to the appropriate package insert for additional information about the **Experiment Information** dialog box.
2. Save the experiment by clicking the **Save** button.
3. Close the **Experiment Information** dialog box.

Priming the Fluidics Station

A prime is necessary to ensure that the wash lines are full of the appropriate buffer and that the fluidics station is ready to process a cartridge. You should prime the fluidics station:

- when you first start the fluidics station, or
- when you change the wash solutions, or
- before processing a cartridge if you have performed a shutdown on any module, or
- if the LCD window instructs you to run a prime.

1. Check to ensure that all the wash lines are in the appropriate wash bottles. Please consult the probe array package insert that came with the cartridge kit for the appropriate wash buffer solutions, or contact your Affymetrix Technical Support representative.
2. Remove any loaded cartridges and observe for possible leaks since the system will not report errors during the Prime protocol.
3. If it is not already open, click **Fluidics** under the **Run** menu on the computer workstation.

   The **Fluidics Station** dialog box appears. If more than one fluidics station is present, select the one to be primed in the drop-down list.
4. In the **Fluidics Station** dialog box, click **Protocol**, and then choose **Prime_450** for each module under the **Protocol** drop-down list (Figure 2.3). Click the Run button for each module to be primed.

   Note that by checking the **All Modules**, you can prime all the modules simultaneously.

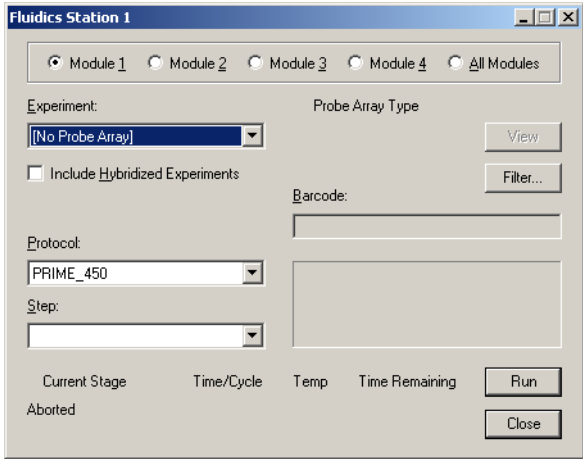

Figure 2.3
Select the protocol from the drop-down list.

5. Follow the instructions in the LCD window as the prime progresses. You must load standard 1.5mL vials in the sample holders of each module that is to be primed. The LCD window on the fluidics station and the **Fluidics Station** dialog box will indicate the status of the prime and when priming is completed.

Washing and Staining the Probe Array

After you have primed the fluidics station, it is ready to wash and stain a sample.

1. If it is not already open, click **Fluidics** under the **Run** menu on the workstation.

   The **Fluidics Station** dialog box appears. If more than one fluidics station is present, select the appropriate instrument from the drop-down list.

2. In the **Fluidics Station** dialog box, select the module that is ready for use and choose the experiment name in the drop-down **Experiment** list. Check to ensure that the experiment name matches the sample and cartridge to be run. If you have barcode reading capability, you can automatically fill in the experiment information using the barcode read/write feature.

3. In the **Protocol** drop-down list, choose the protocol that matches the cartridge type (Figure 2.4).

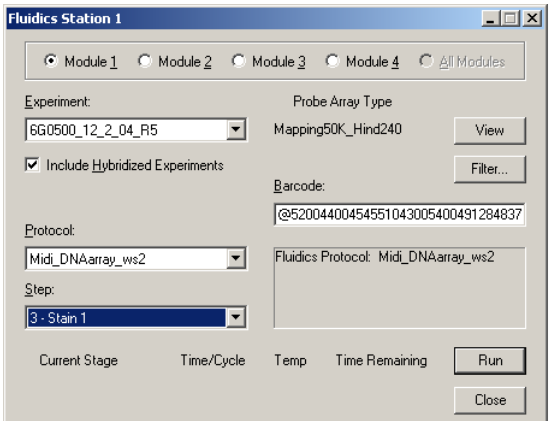

Figure 2.4
Select the protocol from the drop-down list.

NOTE

You can also choose a customized hybridization-wash or wash protocol here. Refer to the GCOS user's guide or to *Customizing the Protocol* on page 32. If you are running a customized protocol, check the parameters of each of the protocols chosen to be sure they are appropriate for your experiment. This can be done in the *Fluidics Protocol* dialog box found by choosing *Edit Protocol* under the *Tools* menu.

4. Click the **Run** button to begin hybridization of the sample.

   The protocol will begin. The LCD window on the fluidics station and the **Fluidics Station** dialog box on the workstation terminal graphic user interface (GUI) will indicate the status of the hybridization as it progresses.

5. Follow the instructions on the LCD window or in the **Fluidics Station** dialog box as displayed in the workstation terminal GUI. A selection of the available prompts is given below as examples:

a. If prompted to "**Remove Vials**," remove the vials from the sample holder of the fluidics station.

b. If prompted to "**Load Cartridge**," open the cartridge holder by pressing down on the cartridge lever to open the cartridge loading door.

Place the appropriate cartridge into the cartridge holder corresponding to the module set up in the experiment. See Figure 2.5.

Figure 2.5
Inserting the cartridge into the cartridge holder - note orientation and probe array label

Flip the cartridge lever up to engage the cartridge septa needles into the septa. Proper engagement of the washblock with the cartridge is indicated by a change in the message on the LCD (Figure 2.6).

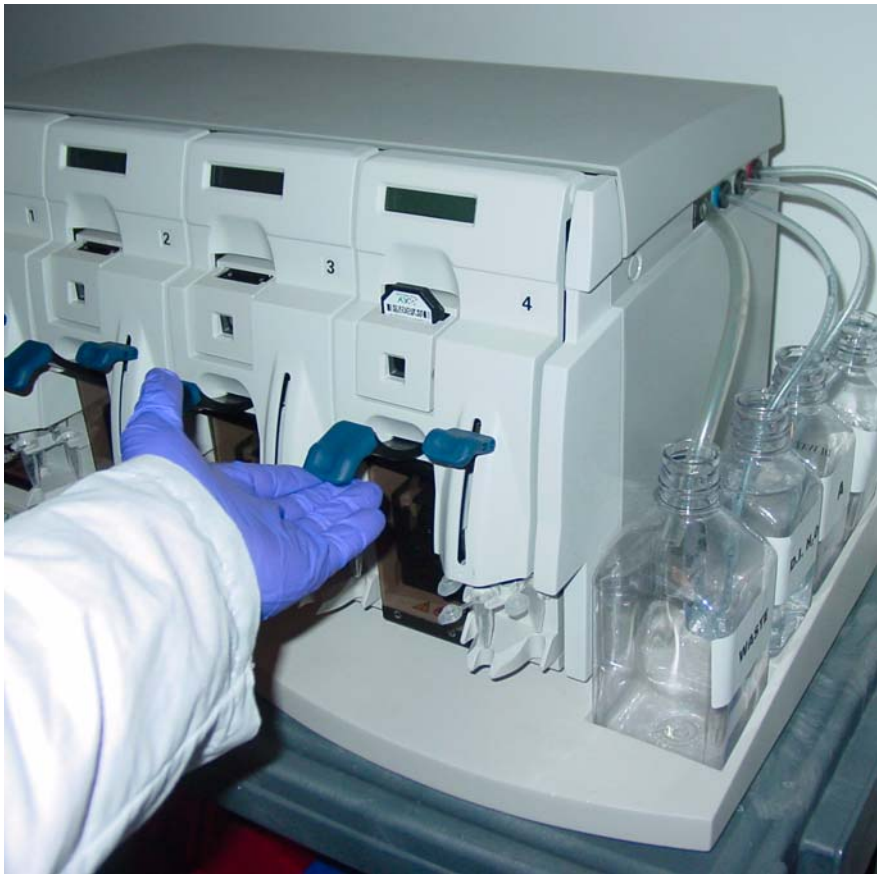

Figure 2.6
Flip the cartridge lever up to engage the cartridge septa needles into the septa.

CAUTION

To minimize damage to the probe array, the door closure forces are controlled. If proper engagement does not occur, simply press on the washblock cartridge door to complete the action.

DO NOT FORCE UP THE CARTRIDGE LEVER.

c. If prompted to "**Load Vials 1-2-3**," place the three 1.5mL vials containing the proper reagents into the sample holders 1, 2, and 3 on the fluidics station. For example, if you are using the streptavidin-anti-streptavidin-biotinylated-antibody-SAPE staining protocol for the E. coli Genome Array, follow this procedure (Figure 2.7).

a Place one vial containing the streptavidin solution mix in sample holder 1.

b Place one vial containing the anti-streptavidin

biotinylated antibody in sample holder 2.

c Place one vial containing the streptavidin phycoerythrin (SAPE) solution in sample holder 3.

NOTE

If you are staining certain eukaryotic targets, you will use SAPE in vials 1 and 3.

If you are using a protocol that requires only a single stain (such as for other eukaryotic targets), you will be prompted to place one vial at vial holder position 1.

Some genotyping scripts may require SAPE in vial 1, anti-streptavidin in vial 2 and an MES holding buffer in vial 3.

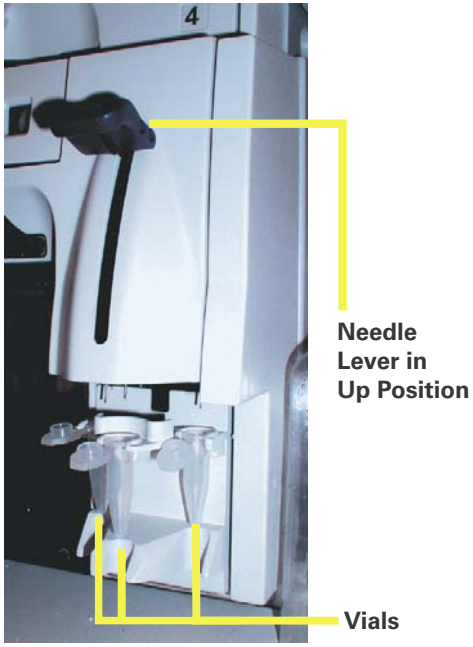

Figure 2.7
The samples vials on the sample holder with the needle lever up — note the orientation of the vial caps.

NOTE

When you place the vials into the holders, orient the vial caps toward you so that the vials seat snugly into their respective holders.

d. When you have loaded the vials, gently but firmly press down on the needle lever to insert the needles into the vials. The run will commence automatically. See Figure 2.8 and Figure 2.9.

Figure 2.8
Press down on the needle lever to begin the run.

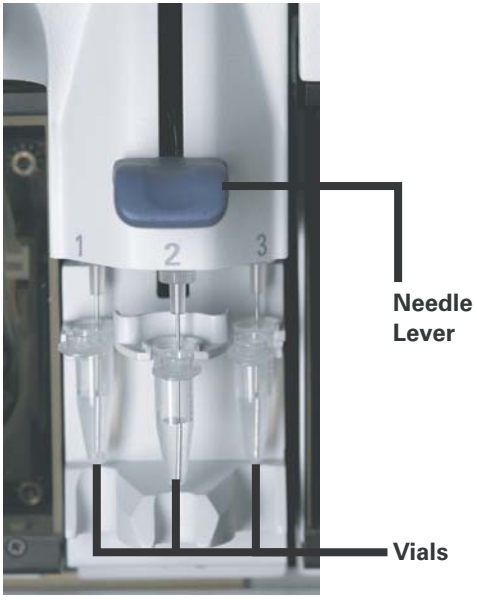

Figure 2.9
The vials on the sample holder with the needle lever down- note the orientation of the vial caps.

As the staining run progresses, check to ensure that the cartridge is filling properly and that bubbles are not forming. If it is not filling properly, see the note below in this chapter.

6. When the staining and washing are complete, the LCD window should display the following:

Eject Cartridge

7. Eject the cartridge by pushing down on the cartridge lever. The LCD window should display the following:

Engage Washblock

NOTE If bubbles are present in the cartridge, return it to the cartridge holder. Engage the cartridge by flipping up the cartridge lever to the closed position.

The fluidics station will drain the cartridge and then fill it with a fresh volume of the last wash buffer used. When it is finished, if the LCD window displays **EJECT CARTRIDGE** again, remove the cartridge and inspect it again for bubbles. If no bubbles are present, it is ready to scan. Proceed to step 8.

If you have made ten attempts to fill the cartridge without bubbles and have been unsuccessful, the fluidics station will no longer display **EJECT CARTRIDGE** after refilling the cartridge. Instead, it will terminate with the prompt **DO CLEAN CYCLE**. Remove the cartridge and run the Cleanout protocol on the particular module. Fill the cartridge manually with the last buffer used by inserting a pipette tip or syringe needle through the bottom septum and by using a second pipette tip or syringe needle in the top septum to permit air to escape.

Refer to ***Troubleshooting and Assistance on page 49*** for possible causes and solutions to bubbles appearing in the cartridge.

IMPORTANT **If you are using a script with the MES holding buffer, you must manually remove and refill the probe array cartridge.**

8. Pull up on the cartridge holder lever to re-engage the wash block.
9. Lift up on the needle lever to remove the needles from the vials.
10. Replace the used vials with new empty vials.
11. Press down on the needle lever.

    The fluidics station will automatically perform a Cleanout protocol. The LCD window will indicate the progress of the Cleanout protocol. When the Cleanout protocol is complete, the LCD window should display the following:

Remove Vials

12. Lift the needle lever and remove the sample vials from the sample holder.

Customizing the Protocol

You must modify a protocol with the new parameters before you can run it on the fluidics station. Protocol changes will not affect runs in progress. For more specific instructions, refer to the GCOS user's guide or contact your Affymetrix Technical Support representative.

1. Select **Edit Protocol** from the **Tools** menu on the workstation.

   The **Edit Protocol** dialog box appears.

2. In the **Edit Protocol** dialog box under **Protocol Name**, click the arrow to open a list of protocols. Click the protocol to be changed.

   The **Protocol Name** text box displays the protocol name. The conditions for that protocol are displayed on the right side of the **Edit Protocol** dialog box.

3. Select the item to be changed and enter the new parameters as needed. The parameters must be within the ranges shown below:

Parameter	Valid Range
Hybridization or Stain Time (Seconds)	0 to 86,399
Temperature (°C)	15 to 50
Number of Wash Cycles	0 to 99
Mixes per Wash Cycle	1 to 99

NOTE

Enter 0 (zero) for the hybridization time if you desire only a wash. Enter 0 (zero) for the number of wash cycles for a wash solution that you will not use.

4. To return to the default values for the protocol selected, click the **Defaults** button.
5. Once you have modified all the protocol conditions, click the **Save** button to save the modified protocol. To save the modified protocol

under a different name, enter the new name in the **Protocol Name** box before clicking the **Save** button.

6. Click the **Close** button to leave the dialog box.

Shutting Down

You should perform the Shutdown protocol at the end of a session. Do not keep the fluidics station on if you will not use it again within the next 12 hours. This will reduce the risk of salt buildup in the instrument.

IMPORTANT !

To maintain the cleanliness of the fluidics station and obtain the highest quality image and data possible, Affymetrix recommends performing a weekly bleach protocol. Please refer to *Instrument Care and Maintenance* *on page 35* for further details.

1. As with the prime protocol, the shutdown protocol requires three 1.5 mL vials for each module.
2. After removing a probe array from the probe array holder, the LCD window displays the message ENGAGE WASHBLOCK.
3. Engage the washblock by gently pulling up on the probe array lever to the up position.

   The fluidics station automatically performs a Cleanout protocol. The LCD window indicates the progress of the Cleanout protocol.
4. When the fluidics station LCD window indicates REMOVE VIALS, the Cleanout protocol is complete.
5. Remove the vials from the sample holder.
6. If you have completed the fluidics runs, place the wash lines into a bottle filled with deionized water.

IMPORTANT !

To avoid contamination, this DI water should be replaced with fresh DI water before performing a fluidics run.

7. Choose Shutdown_450 for all modules from the drop-down **Protocol** list in the Fluidics Station dialog box. Click the **Run** button for all modules.
8. After the Shutdown protocol is complete, flip the ON/OFF switch to the **OFF** position.

Chapter 3

Instrument Care and Maintenance

Chapter 3

Introduction

This chapter provides instructions on caring for and maintaining the instrument, and on troubleshooting if problems arise.

INSTRUMENT CARE

- Use a surge protector on the power line to the fluidics station.
- Always run a Shutdown protocol when the instrument will be off or unused overnight or longer. This will prevent salt crystals from forming within the fluidics system.
- When not using the instrument, leave the sample needles in the lowered position. Each needle should extend into an empty vial. This will protect them from accidental damage.
- Always use deionized water to prevent contamination of the lines. Change buffers with freshly prepared buffer at each system startup.
- The fluidics station should be positioned on a sturdy, level bench away from extremes in temperature and away from moving air.

IMPORTANT !

Before performing maintenance, turn off power to the station to avoid injury in case of a pump or electrical malfunction.

INSTRUMENT MAINTENANCE

To ensure proper functioning of the fluidics station, you should perform periodic maintenance.

Fluidics Station Bleach Protocol

Affymetrix recommends a weekly cleaning protocol for the fluidics station. This protocol uses commonly purchased sodium hypochlorite bleach.

This protocol is designed to eliminate any residual SAPE-antibody complex that may be present in the fluidics station tubing and needles. The protocol runs a bleach solution through the system followed by a rinse cycle with deionized (DI) water. This protocol takes approximately one hour and forty minutes to complete. Affymetrix recommends running this protocol weekly. You can find the current version of the protocol at

www.affymetrix.com/support/technical/fluidics_scripts.affx.

THE BLEACH CYCLE

To avoid carryover, or cross contamination, from the bleach protocol, Affymetrix recommends the use of dedicated bottles for bleach and DI water. You can obtain additional bottles from Affymetrix.

Table 3.1
Affymetrix recommended bottles

Part Number	Description
400118	Media Bottle, SQ, 500ml
400119	Media Bottle, SQ, 1000ml

1. Disengage the washblock for each module by pressing down on the cartridge lever. Remove any probe array cartridge (Figure 3.1).

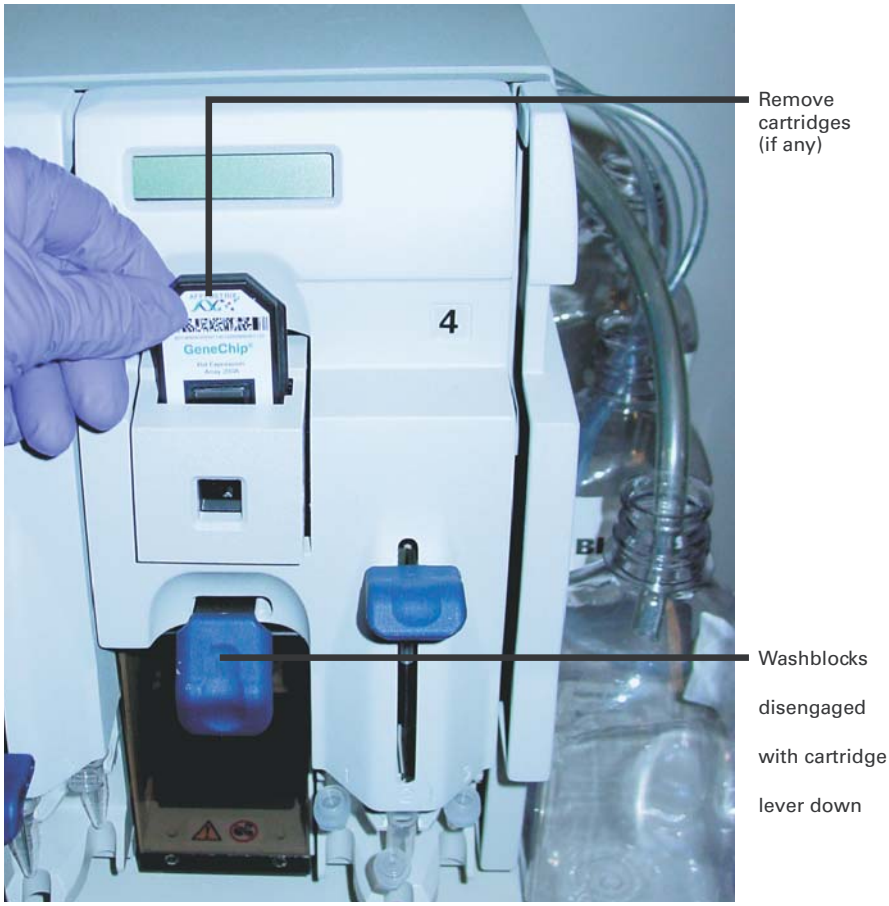

Figure 3.1
Disengaged washblocks showing cartridge levers in the down position, remove any cartridges

2. Prepare 500mL of 0.525% sodium hypochlorite solution using deionized water. For example: follow these directions to make 500mL of bleach.

   In a 1 liter plastic or glass graduated cylinder combine 43.75mLs of commercial bleach (such as Clorox® bleach, which is 6% sodium hypochlorite) with 456.25mLs of DI H2O, mix well. Pour the solution into a 500mL plastic bottle, and place the plastic bottle on fluidics station.

IMPORTANT !

The shelf life of this solution is 24 hours. After this period, you must prepare a fresh solution.

NOTE

Each fluidics station with four modules requires 500mL of the 0.525% sodium hypochlorite solution.

3. Place on the fluidics station an empty one liter waste bottle, a 500 ml bottle of bleach and a one liter bottle of DI water as shown in Figure 3.2. Insert the waste line into the waste bottle (Figure 3.2).
4. Immerse all three wash and water lines of the fluidics station into the 500 ml of bleach solution (Figure 3.2). DO NOT IMMERSE THE WASTE LINE INTO THE BLEACH.

NOTE

The BLEACH protocol requires approximately one liter of DI water.

Figure 3.2
The bleach cycle. Immerse the tubes into the 0.525% sodium hypochlorite solution. The waste line remains in the waste bottle.

5. Open GCOS, Microarray Suite, or the current version of the Affymetrix control software. Click **Run →Fluidics...** from the menu. Alternatively, click the down arrow Protocol list on the toolbar. The protocol window appears (Figure 3.3).

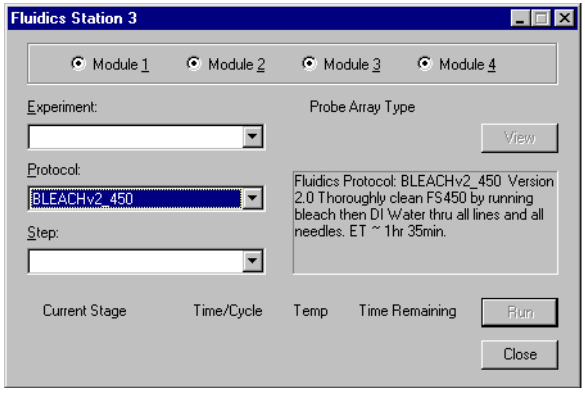

Figure 3.3
The Fluidics Station protocol window: select all modules.

6. Choose the current bleach protocol (in the current example, it is BLEACHv2_450) for each of the respective modules in the Protocol drop-down list. Select all four modules, 1 to 4, and click **Run**. The fluidics station will not start the bleach protocol until you press down on the needle lever (Figure 3.4).

NOTE

Temperature will ramp up to 50°C.

7. Follow the prompts on each of the LCD. Load empty 1.5mL vials onto each module if you have not already done so.
8. Press down on each of the needle levers to start the bleach protocol (Figure 3.4).

Figure 3.4
Press down on the needle levers to start the bleach protocol.

9. The fluidics station will begin the protocol and begin to empty the lines and perform the cleaning cycles using bleach solution.
10. After approximately 30 minutes, the LCD will prompt you when the bleach cycle is over and the rinse cycle about to begin.

THE RINSE CYCLE

Once the bleach cycle has finished, the second part of the protocol is a rinse step. This step is essential to remove all traces of bleach from the system. Failure to complete this step can result in damaged arrays.

1. Follow the prompts on the LCD for each module. Lift up on the needle levers and remove the bleach vials. Load clean, empty vials onto each module.
2. Remove the three wash and water lines from the bleach bottle and transfer them to the DI water bottle (Figure 3.5). At this step, you need not be concerned regarding the bleach that remains in the lines.

Figure 3.5
Immerse the three wash and water lines in the DI water bottle.

3. Press down on the needle levers to begin the rinse cycle. The fluidics station will empty the lines and rinse the needles.
4. When the rinse is completed after approximately one hour, the fluidics station will bring the temperature back to 25°C and drain the lines with air. The LCD display will read CLEANING DONE.
5. Discard the vials employed for the bleach protocol.
6. Follow these suggestions after you have completed the bleach protocol (Table 3.2).

Table 3.2
Quick reference guide to using the FS-450

If you are:	Then do this:
Planning to use the system immediately	After running the bleach protocol, remove the DI water supply used in the rinse phase and install the appropriate reagents for use in your next staining and washing protocol (including fresh DI water). • Perform a prime protocol without loading your probe arrays. **Failure to run a prime protocol will result in irreparable damage to the loaded hybridized probe arrays.**
Not planning to use the system immediately	Since the system is already well purged with water, you need not run an additional shutdown protocol. Just remove the old DI water bottle and replace it with a fresh bottle.
Not planning to use the system for an extended period of time (longer than one week)	Remove the DI water and perform a "dry" protocol shutdown. This will remove most of the water from the system and prevent unwanted microbial growth in the supply lines. Also, remove the pump tubing from the peristaltic pump rollers.

NOTE

After you have completed the bleach protocol, discard the vials.

Peristaltic Tubing Replacement

Periodically the peristaltic tubing requires replacement because of wear, contamination, or in order to avoid salt buildup. Inspect the tubing, if you see evidence of these conditions, follow the procedure outlined below.

IMPORTANT

For systems in routine use, Affymetrix recommends monthly replacement of the tubing. To ensure proper performance, use only tubing available from Affymetrix. This tubing is manufactured to the required specifications to ensure proper fluid delivery and array performance. You can obtain additional tubing by ordering from Affymetrix:

Part Number	Description	Quantity
400110	Tubing, Silicone Peristaltic, 8.5	1

Wear gloves when changing tubing. Do not allow fluid from old tubing to spill onto surfaces.

1. Open the module door (Figure 3.6). Also see item number 2 in Figure 1.1 on page 11.

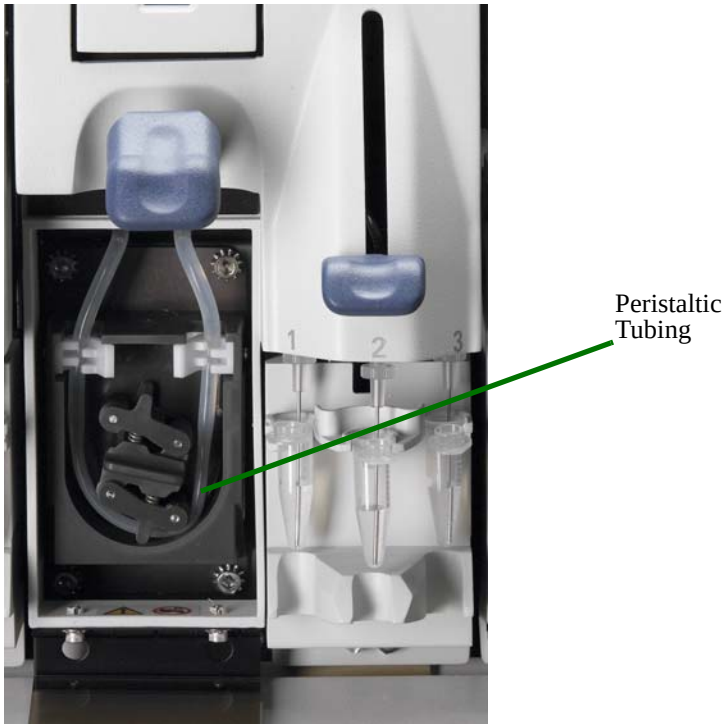

Figure 3.6
Module door open showing peristaltic tubing

2. Open the white clamps to release tubing on both sides. See Figure 3.7.

WARNING

Do not attempt to replace the tubing on a module where the module has been removed from the case of the fluidics station. In this case, rotating the pump may damage the motor driver circuitry.

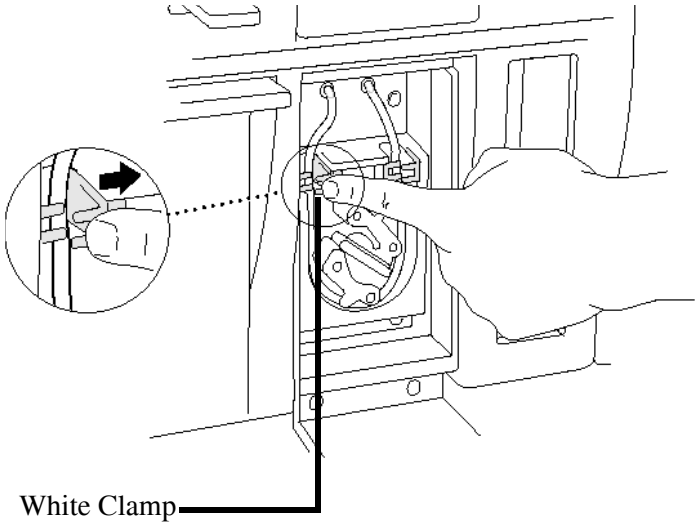

Figure 3.7
Releasing peristaltic tubing

3. Pull tubing off while gently turning the peristaltic pump head. Discard old tubing.
4. Replace tubing with new peristaltic tubing supplied with the accessory kit as described below:
    a. Attach one end of the new tubing to the fitting on the right at the top of the pump enclosure.
    b. Insert the tubing into the clamp under the fitting without stretching the portion of the tubing between the fitting and the clamp. There should be a small amount of slack in that portion of the tubing.
    c. Work the tubing into the pump head while slowly turning the pump.
    d. Insert the free end of the tubing into the other clamp, and attach it to the other fitting.
    e. Close the drop-down module door.
5. Order more replacement tubing (P/N 400110).

Troubleshooting and Assistance

If problems arise with the fluidics station, use the following tables to locate the description that matches the problem. If you cannot find a solution, call Affymetrix Technical Support for assistance.

TROUBLESHOOTING DECISION TREE

The following simple flow charts (Figure 3.8 and Figure 3.9) show you how to begin troubleshooting the FS450/250 for a Missing Fluid Error (MFE).

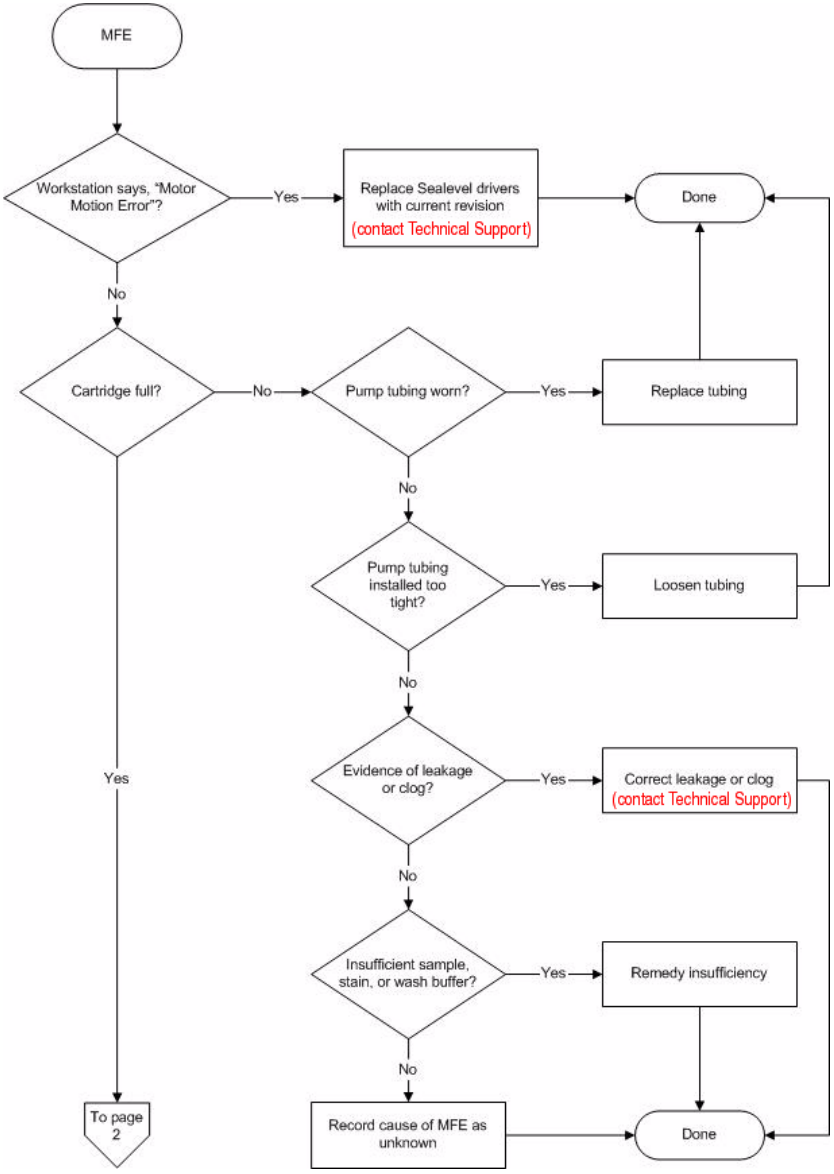

Figure 3.8
Troubleshooting decision tree, page 1

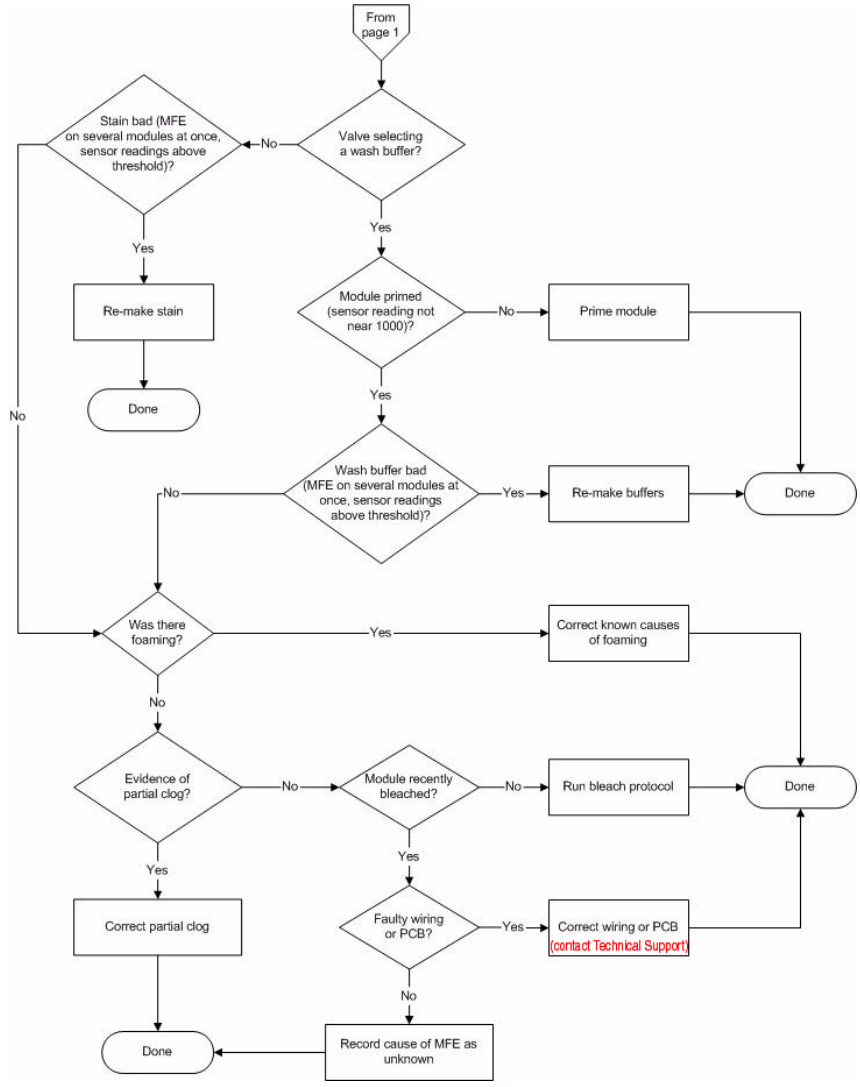

Figure 3.9
Troubleshooting decision tree, page 2

PROBLEMS AND SOLUTIONS

Table 3.3
Common error messages, their meanings, probable causes and solutions

Error Message	Problem	Possible Cause	Solution
Missing Fluid Error	Cartridge not filling completely with sample solution or buffer during initial stages of hybridization wash or staining protocol.	Possible holes in the septa of the cartridge.	Run Recover script, and then use another cartridge.
		Sample or staining solution not in place properly.	Run Recover script. Make sure sample or stain vial is in the sample holder.
		Insufficient volume of sample or staining solution (500µL).	Run Recover script. Add more sample solution to the sample vial.
		Blocked sampling tube or line of the fluidics station.	Run Recover script. Run the Clean or Prime script with fresh deionized (DI) water to flush out salt blockage.
		Failure of one of the fluidics sensors.	Call Affymetrix Technical Support for service.
		Pump tubing stretched too tightly around the pump.	Loosen the tubing clamps, allow tubing to relax, close the clamps.
	Cartridge not filling completely with buffer during wash script	Buffer bottle empty.	Fill buffer bottles.
		Module not primed.	Prime module.
	System detects improper conditions while filling. Note where in protocol error occurred.	• Missing or insufficient stain or antibody in vial? • Wash empty? • Air bubbles in line? • Leaks?	Identify if chip is filled • If important to recover fluid in chip, and then run **Recovery** script, followed by **Resume** function • If not important to recover fluid in chip, run **Resume** function

Table 3.3
Common error messages, their meanings, probable causes and solutions

Error Message	Problem	Possible Cause	Solution
	Recovered less sample than initial input during Recover script.	Loose tubing attachments inside the fluidics station.	Call Affymetrix Technical Support for service.
Fluidics Station X Does Not Respond		Power not switched on at the fluidics station. Incorrect fluidics station designated for communication. Loose cables.	Turn fluidics station power on, and then try to connect again. Designate correct fluidics station on workstation. Firmly connect cables to fluidics station.
Sensor Timeout	"Sensor Timeout" error message on workstation.	No user response to "Remove Vial" prompt or other prompt.	Start the selected script again.
Error While Draining Error While Filling	Cartridge is not filling or draining properly.	Defective septa in cartridge. Insufficient sample or stain volume. Excessive bubbling in cartridge. Buffer conductivity too low. Failure of one of the fluid sensors.	Use a new cartridge. Add more sample solution to sample vial. Change the buffer: reduce detergent. Change the buffer: increase salt. Call Affymetrix Technical Support for service.

Table 3.3
Common error messages, their meanings, probable causes and solutions

Error Message	Problem	Possible Cause	Solution
Error While Filling	System detects improper conditions while filling. Note where in protocol error occurred.	• Missing or insufficient stain or antibody in vial? • Wash or DI water empty? • Air bubbles in line? • Leaks?	Identify if chip is filled: • If important to recover fluid in chip, and then run **Recovery** script, followed by **Resume** function • If not important to recover fluid in chip, run **Resume** function
Invalid Command	Communications error detected Note where in protocol error occurred		Identify if chip is filled. • If important to recover fluid in chip, and then run **Recovery** script. • Attempt to rerun script if sample loss can be tolerated. If problem persists, contact Affymetrix for service • If sample loss cannot be tolerated, do not attempt to rerun script. Contact Affymetrix for service
Temperature Timeout	Temperature does not reach specified temperature.	Temperature has not reached required level in expected time if ambient temperature is within operating specifications (15 – 30 degrees C).	Call Affymetrix Technical Support for service.
Improper Script	Script does not work.	User is attempting to run a FS400 script on FS450Dx	Download proper FS450Dx script and continue

Table 3.3
Common error messages, their meanings, probable causes and solutions

Error Message	Problem	Possible Cause	Solution
Valve Motion Error			Run Home script and run desired script again If problem persists, contact Affymetrix for service.
Valve Not Homed			Run Home script and run desired script again If problem persists, contact Affymetrix for service.
Valve Out of Position			Run Home script and run desired script again If problem persists, contact Affymetrix for service.

MEANING OF ERROR MESSAGES

The following lists some of the common error messages and what they mean (Table 3.4).

Table 3.4
Common error messages

Error Message	Meaning
"Invalid Command"	The script contains a command that can not be executed because its command code is either undefined or has a format error.
"Improper Script"	The first command of the script is not the required FS450 command.
"Temperature Timeout"	The Re-attempt command timed out before the set point temperature was reached.
"Sensor Timeout"	The Await Sensors command timed out before the anticipated sensor pattern was seen.
"Valve not Homed"	The Home command did not result in the valve reaching it HOME position.
"Valve Motion Error"	The Valve command did not result in the valve reaching it target valve position.
"Valve out of Position"	According to the outer valve encoder, the valve did not reach a valid position when it was last rotated.
"Error while Filling"	While filling the cartridge, the AwaitMotor command terminated because of the step count not the expected sensor pattern, and that the same error had occurred several times consecutively.
"Error while Draining"	While draining the cartridge, the AwaitMotor command terminated because of the step count not the expected sensor pattern, and that the same error had occurred several times consecutively.

Table 3.4
Common error messages

Error Message	Meaning
"Missing Fluid Error" Examples: "Stage C" "WashA" "Sense/Threshold" "960/890"	"Stage C" "WashA" "Sense/Threshold" "960/890" The Pump command completed its step count before the conductivity sensor determined that the cartridge contained a solution with conductivity below the set threshold value. The Missing Fluid Error (MFE) Display not only gives a visual notification of an error condition to the operator, but gives the operator information that enables him/her to determine the cause of the error. It does this by displaying information about the sensor value and the fluid that caused the error. It shows this internal information in a continuous loop until the machine is powered down or a script is started. For example: Missing Fluid Error for 4 seconds Stage A valvePos WashA for 4 seconds Sense/Threshold 820/600for 4 seconds

Other Problems and Solutions

Table 3.4 lists other problems, causes and solutions that you may encounter.

Table 3.5
Other problems

Problem	Possible Cause	Solution
Air bubbles left in cartridge at the end of a hybridization-wash script.	Air bubble in wash line	See *Washing and Staining the Probe Array* on page 21.
Buffer leaking inside the fluidics station.	Loose tubing attachments inside the fluidics station. Washblock requires replacement. Salt buildup in the lines of the fluidics station.	Call Affymetrix Technical Support for service. Call Affymetrix Technical Support for service. Run the Clean or Prime script with fresh DI water to flush out salt blockage.
Cartridge needles of the fluidics station not engaging with the cartridge.	Possible defective septa on the cartridge. Extra flashing on the cartridge. Salt buildup on the cartridge needles. Cartridge holder aligned and attached to the fluidics station improperly. Cartridge holder not properly engaged to the fluidics station.	Use another cartridge. Use another cartridge, or call Affymetrix Technical Support for service. Run the Clean script with fresh DI water to flush out salt blockage. Clean cartridge needles with a wet cotton swab. Call Affymetrix Technical Support for service. Place the cartridge into the cartridge holder. Push the holder door shut, and firmly lift the lever to engage the cartridge needles.

Table 3.5
Other problems

Problem	Possible Cause	Solution
Sample needles do not properly enter vial.	Bent sample needle User may be pressing the needle lever down to quickly or with too much force.	Replace sample needle. Engage sample needle lever more slowly and/or with less force.

When to Contact Technical Support

Under any of the following conditions, unplug the instrument from the power source and contact Affymetrix Technical Support:

- when the power cord is damaged or frayed;
- if any liquid has penetrated the instrument;
- if, after service or calibration, the instrument does not perform to the specifications stated in *Instrument Specifications on page 81*.

If the instrument must be returned for repair, call Affymetrix Technical Support.

IMPORTANT **!**

Make sure you have the model and serial number.

Affymetrix, Inc.
3380 Central Expressway
Santa Clara, CA 95051
USA

E-mail: support@affymetrix.com
Tel: 1-888-362-2447 (1-888-DNA-CHIP)
Fax: 1-408-731-5441

Affymetrix UK Ltd

Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH
United Kingdom

E-mail: supporteurope@affymetrix.com
Tel: +44 (0) 1628 552550
Fax: +44 (0) 1628 552585

Affymetrix Japan, K. K.

Mita NN Bldg
16 Floor, 4-1-23 Shiba,
Minato-ku, Tokyo 108-0014
Japan

Tel: (03) 5730-8200
Fax: (03) 5730-8201

Appendix A

Customer Module Replacement Procedure

Appendix A

Introduction

This section describes the procedure to replace a Fluidics Station 450 or 250 module in the field.

CAUTION

Warning: To ensure user safety, avoid contact with any internal components in the instrument base while removing or installing modules unless specifically indicated by these instructions.

MODULE REMOVAL

The procedure below shows you how to remove a module. You can also refer to quick reference card, P/N 08-0099, *Fluidics Station 450/250 Module Replacement Procedure*.

Tools Needed: Standard flathead screwdriver

CAUTION

When making connections, always turn off power to all devices in the chain. To avoid electrostatic discharge and damage to sensitive electronic components in the modules, touch the grounding lug under the top cover before proceeding.

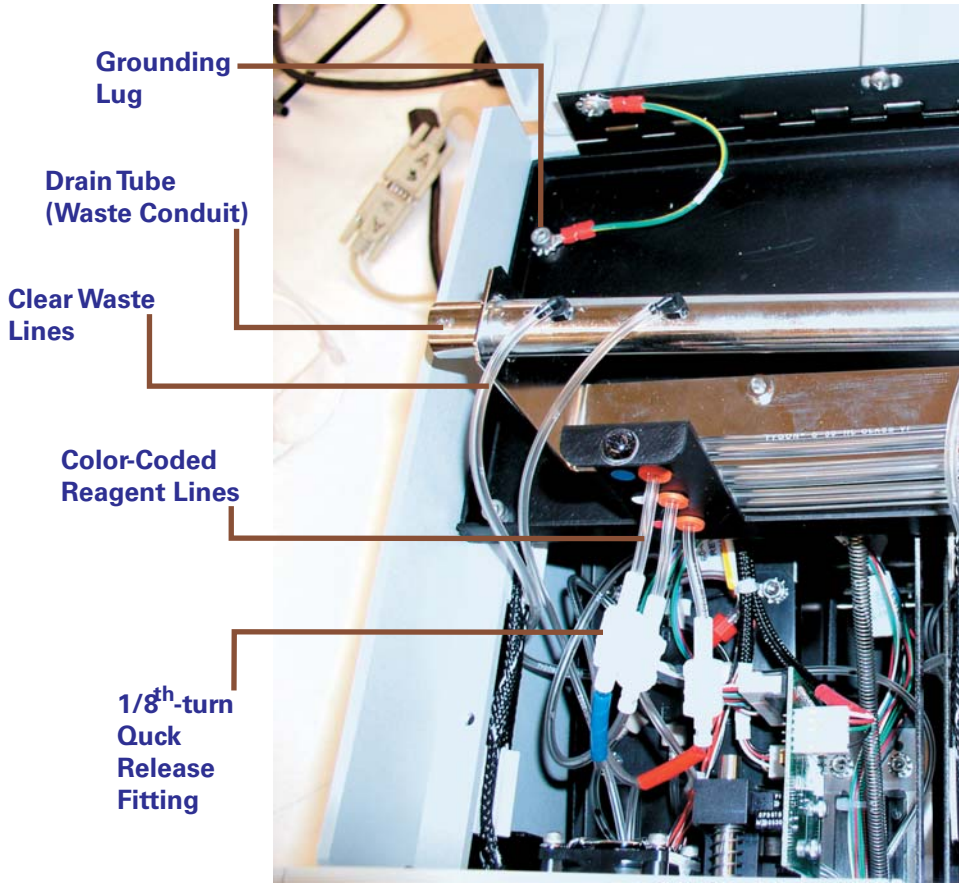

Figure A.1
Components under the fluidics station cover. Note that in some of the newer FS-450 versions, the side buttons may no longer be present and the drip tray and supply lines may be different.

1. Open top cover by pressing the two buttons located on the top left and right sides towards the front of the instrument. See Figure 1.1 on page 11 and Figure A.1. In the newer FS-450 versions, the buttons are no longer present. In this case, simply lift the lid.
2. Disconnect the module's color-coded reagent lines (wash-A, wash-B, and DI) from the lines coming from the manifold using the 1/8th turn,

quick-disconnect fittings, and tuck the lines into the space around the eight-way valve. See Figure A.2.

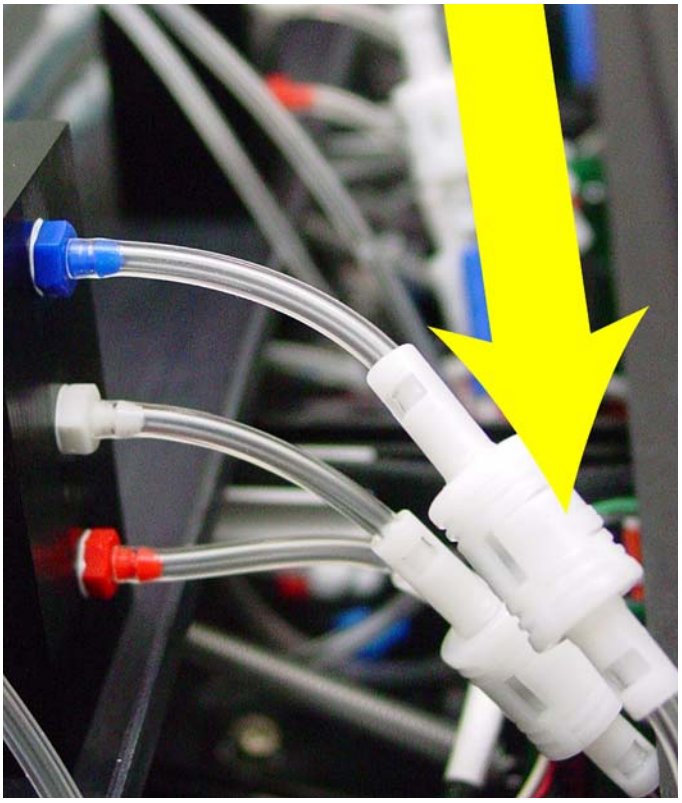

Figure A.2
Disconnect the color-coded reagent line tubes

3. Open the software and run the DRAIN_450 script until completion.

IMPORTANT !

The DRAIN script pumps the residual liquid in the reagent lines out to waste to reduce the probability that a droplet of buffer will fall from the disconnected ends of the lines onto one of the PC boards.

4. When the Drain_450 script is finished, disconnect the clear waste lines from the drain tube (also called the waste conduit, or waste trough. This

is the large chrome-angled tube running along the back). See Figure A.3.

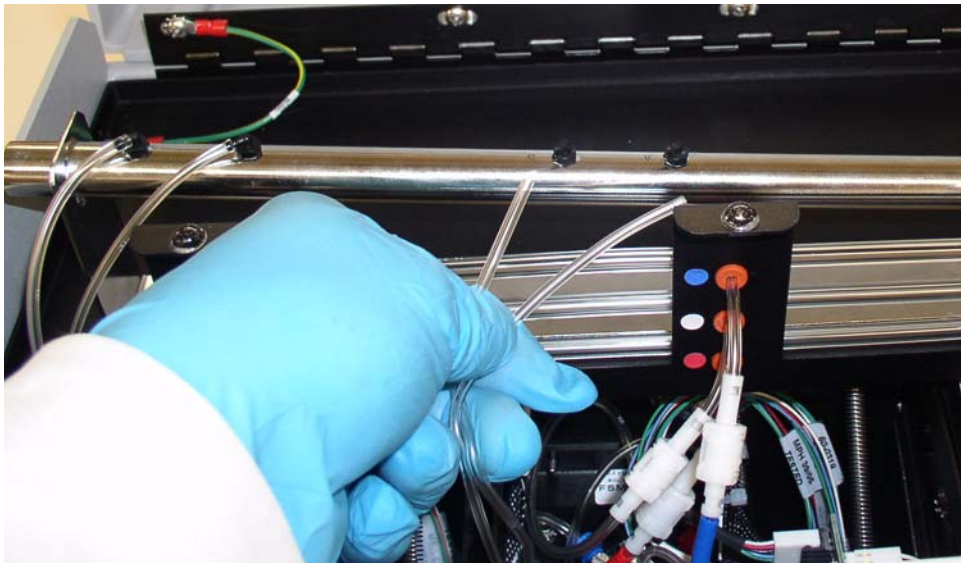

Figure A.3
Disconnect the clear, waste line tubes from the waste line.

5. Ensure that no scripts are running on the supporting workstation and no scripts are running on the FS450 for which the module replacement is required.
6. Turn off power to the FS450 (the ON/OFF switch is located at the lower left hand side of instrument). Verify the power is OFF by observing that the LCD backlight has shut off.
7. Place the packaging containing the new module on a sturdy, flat surface and remove the new module.
8. Inspect the new module for any damage caused by shipping. If OK, place module on a sturdy, flat surface near the FS450.
9. Push all tubing into the module. Avoid snagging when the module is removed
10. Using a slotted screwdriver (also called flathead, standard, or minus screwdriver), slowly unscrew the module retaining screw located at the

base of the module, between the vial holder and pump door. See Figure A.4 and Figure A.5.

IMPORTANT !

Take care not to strip the retaining screw. This screw is captive on the module panel; you cannot remove it from the panel. You must gradually pull the module out toward you as you turn the screw otherwise you will strip the screw. Do this in reverse when installing a module.

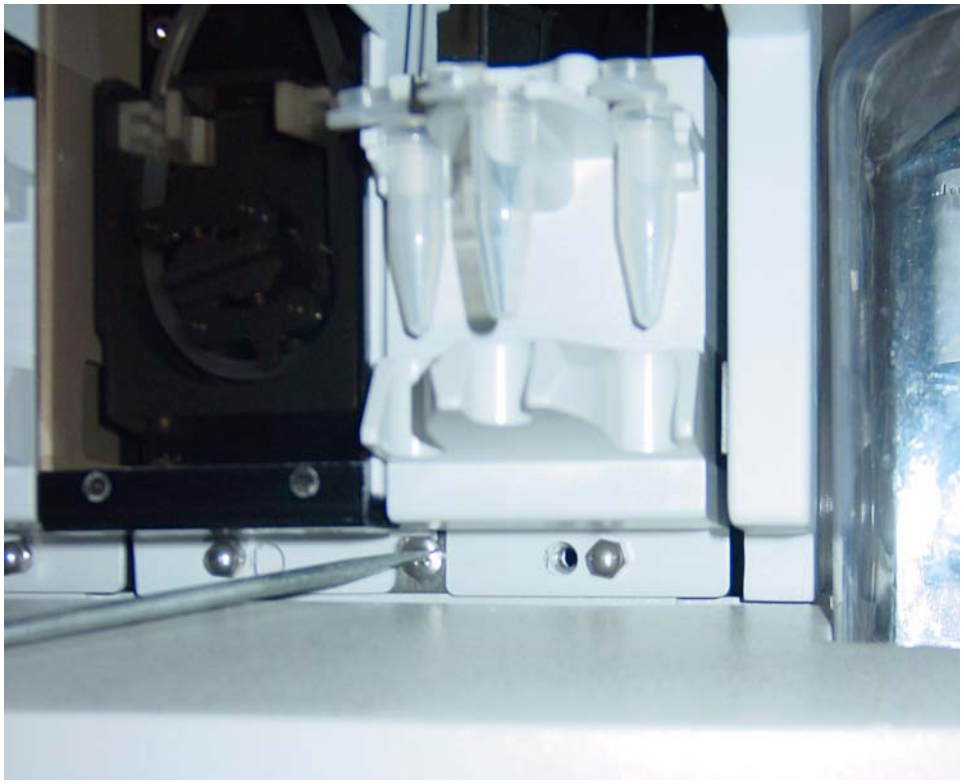

Figure A.4
Remove the module screw at the base of the module.

11. Using both hands as shown below, grab the top and bottom of the module and pull it forward to remove it from the base. As the module is removed, ensure that no tubing attached to the module or base is pinched. See Figure A.5.

Figure A.5
Grasp the module using both hands as shown and gently pull module out.

12. Place the defective module in the new module shipping packaging for return to Affymetrix. Instructions for returning the module are contained within the package.

13. To ensure safety, avoid contact with any internal components in the base.

New Module Installation

The procedure below shows you how to install a new module.

1. Holding the new module in a secure manner, position the module such that it straddles the exposed rails in the base. See Figure A.6.

Figure A.6
Insert a new module and push gently in.

Firmly but gently slide the module back into the base. Carefully align the module but do not attempt to force it flush with the other modules. Lift up the all connectors and tubing coming from the bulkhead on the

base to avoid pinching. Ensure module tubing is not in a position to be pinched. See Figure A.7.

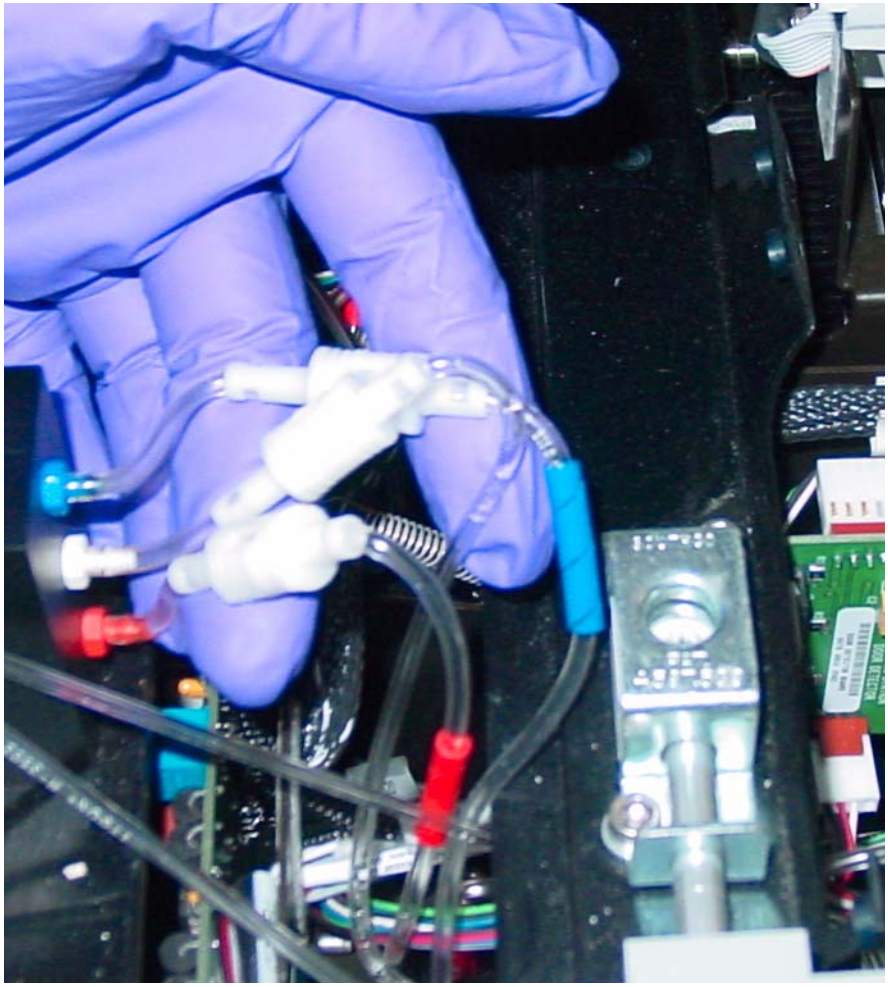

Figure A.7
Ensure that the module tubing will not be pinched by the new module.

2. Reconnect the clear, waste line tubing to the drain tube. See Figure A.8. Connect the waste line tubing from the cartridge to the drain tube inlet, “C” (marked as C on the drain tube) and connect the waste line tubing from the valve to the drain tube inlet “V” (marked as V on the

drain tube). See Figure A.9 and Figure A.10 for waste line tubing identification and inlet locations.

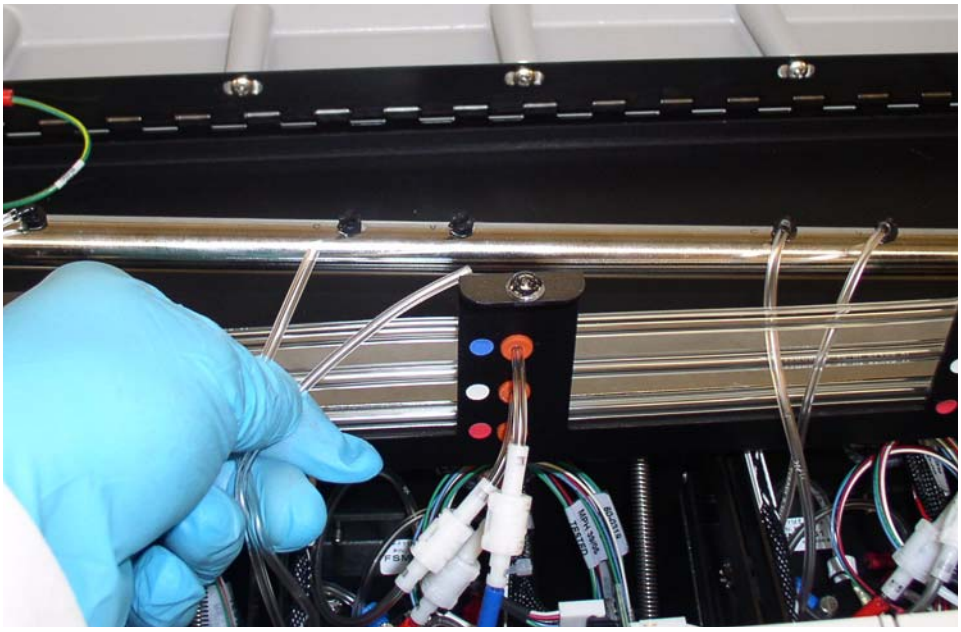

Figure A.8
Reconnect the clear, waste line tubes from the module to the drain tube.

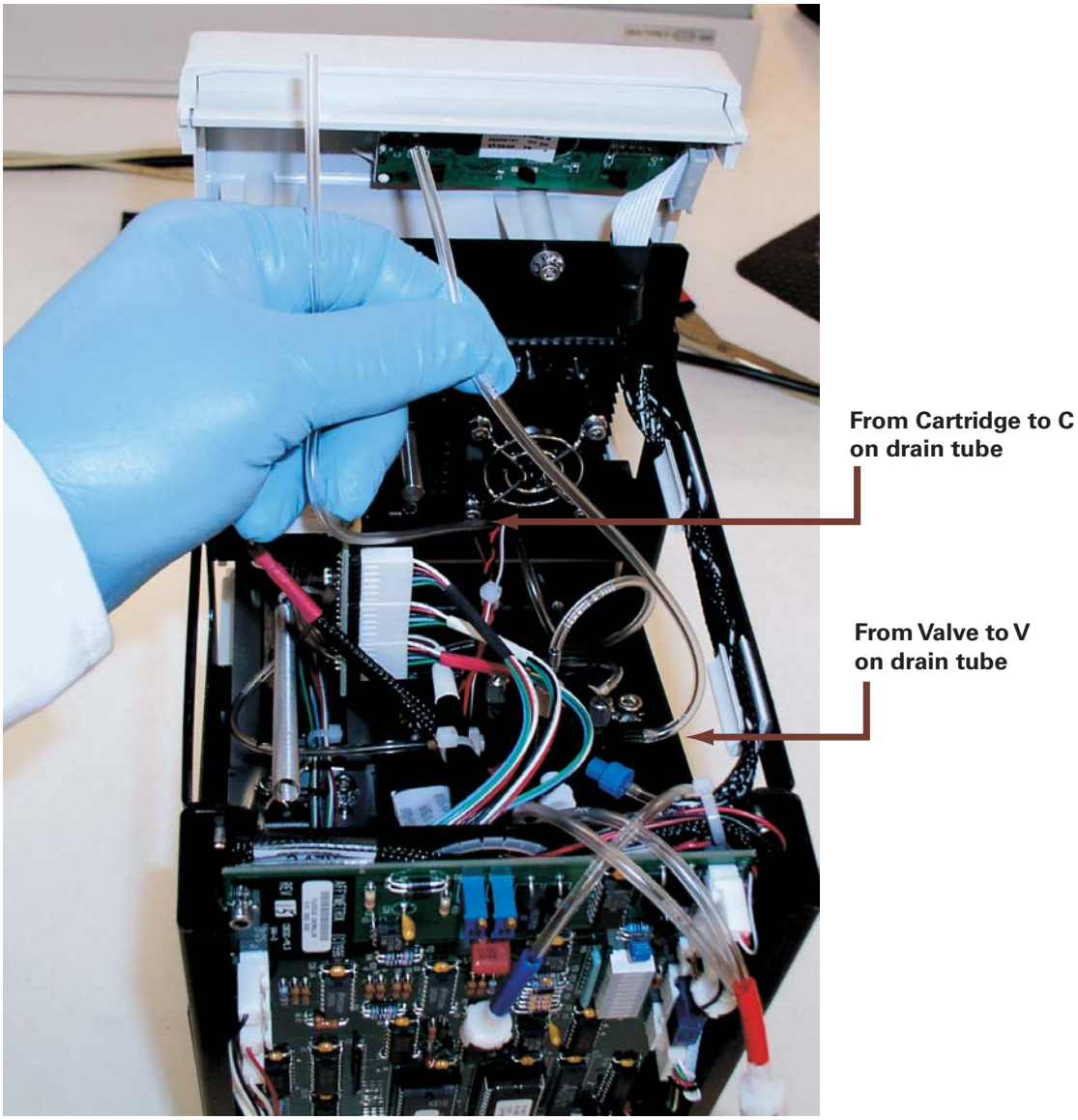

Figure A.9
The “C,” or cartridge, waste line tubing and the “V,” or valve, waste line tubing

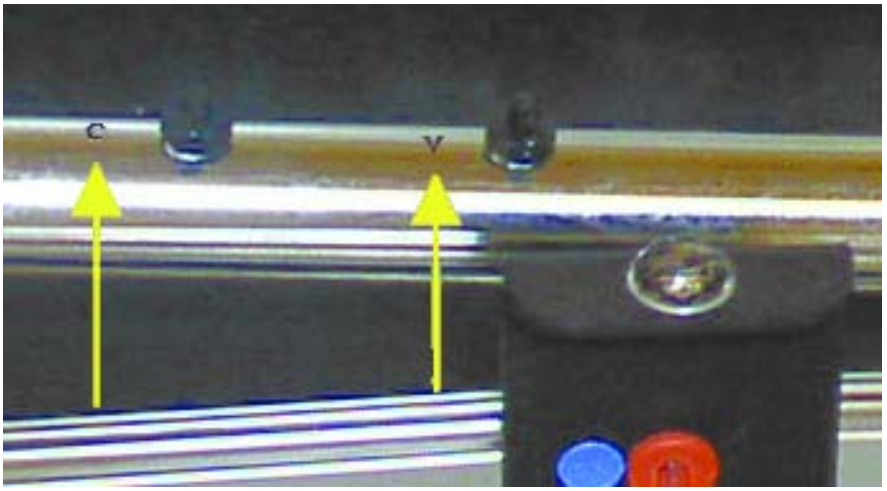

Figure A.10
The locations of the C inlet and V inlet

3. Align the screw at the bottom of the module into the threaded hole on the base. Using a slotted screwdriver, slowly rescrew the module retaining screw until the module is flush with the other modules. See Figure A.4 and Figure A.5.

IMPORTANT !

Important: Take care not to strip the retaining screw. This screw is captive on the module panel. You must gradually work the module in as you turn the screw otherwise you will strip the screw.

4. Carefully lift out the module tubing and connect the three color-coded 1/8th turn fittings that attach the module to the reagent lines.
5. Connect the two clear waste tubes that attach to the waste line by pushing them over the barbed fittings.
6. Close top cover.
7. Turn power **ON**.
8. LCD for new module will indicate "**Power-on Done Not primed**."
9. The Fluidics station is now ready for normal operation.

Appendix B

Using More Than One Fluidics Station

Appendix B

Using one computer workstation, you can connect up to eight fluidics stations together. Follow these steps to connect more than one fluidics station to the computer workstation.

CAUTION

When making connections, always turn off power to all devices in the chain. Failure to do so can cause loss of information.

SETTING THE ADDRESS

Setting the address on the fluidics stations will enable the computer to recognize and correctly control each of the fluidics stations. Each fluidics station connected to a given workstation must have a unique address. You must assign addresses consecutively starting with 1. It is preferable, though not necessary, to number the fluidics stations in the order in which they are connected.

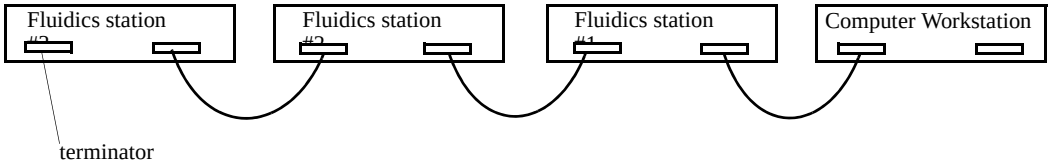

Figure B.1
Addressing fluidics stations consecutively

1. Use a pen or pencil to set each of the three DIP switches on the back of the fluidics station to the ON or OFF position, according to the desired address. Push up for ON and down for OFF.

   All three DIP switches need to be set. Use the following table as a guide (Table B.1).

Table B.1
Guide to setting DIP switches*

Left	Middle	Right		DIP
ON	ON	ON	=	1
ON	ON	OFF	=	2
ON	OFF	ON	=	3
ON	OFF	OFF	=	4
OFF	ON	ON	=	5
OFF	ON	OFF	=	6
OFF	OFF	ON	=	7
OFF	OFF	OFF	=	8

**The FS450 only reads the station address switch when power is first switched on. If the address is changed while the station is already powered on, the new address will not take effect until the station is turned off and then turned back on again.*

CONNECTING FLUIDICS STATIONS TOGETHER

1. Insert the plug end of a nine-pin serial cable into the serial board on the workstation. Use an adapter if required.
2. Insert the other end of the cable into the **CommLink In** nine-pin connector on the back of the first fluidics station.
3. Insert a second nine-pin serial cable into the **CommLink Out** (socket) nine-pin connector on the back of the first fluidics station.
4. Insert the other end of the cable into the **Comm Link In** nine-pin connector on the back of the second fluidics station.
5. Connect the remaining fluidics stations in a chain following the above pattern.
6. Insert a terminator plug into the **Comm Link Out** (socket) nine-pin connector on the back of the last fluidics station in the chain.

Appendix C

Specifications

Appendix C

Introduction

INSTRUMENT SPECIFICATIONS

Fluidics Station Dimensions:

(height, depth, width)
40.2 x 41.0 x 71.1 cm or 15 13/16 x 16 1/8 x 28 inches

Product Weight:

Approximately 80 pounds or 36.3 kg

Power Input:

100 to 240 V**~,** 3 A
300 watts or less
Main supply voltage fluctuations not to exceed 10% of the nominal supply voltage.

Temperature:

Operating: 15° to 30°C
Storage (non-operating):-10° to 60°C

Humidity:

Operating: 10-90% RH, non-condensing
Storage (non-operating):10% to 95% RH

Other:

Pollution degree, 2
Installation category, II

Electrical Supply

The electrical supply shall meet the input specified on the instrument label. Voltage fluctuations shall not exceed 10% nominal supply voltage.

Altitude

<2000m

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Appendix D

Warranty

Appendix D

Warranty

The Affymetrix® GeneChip® Fluidics Station 450 and 250 are warranted to the buyer by Affymetrix. Please refer to the Affymetrix Terms and Conditions received with this instrument at time of sale for information on the warranty.

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Appendix E

Parameters and Messages

Appendix E

Introduction

This section describes the user alterable parameters and the LCD script messages and their meaning.

USER-ALTERABLE PARAMETERS

The following list shows the various parameters that you can change in configuring the fluidics station for a 4 wash staining (Table E.1).

Table E.1
List of user-alterable parameters

Parameter Name	Description	Parameter Values		Default
AWashTemp	Wash A1 Temperature (°C)	15	50	25
AWashCycles	Number of Wash A1 Cycles	0	99	10
AWashMixes	Mixes per Wash A1 Cycle	1	99	2
BWashTemp	Wash B Temperature (°C)	15	50	50
BWashCycles	Number of Wash B Cycles	0	99	4
BWashMixes	Mixes per Wash B Cycle	1	99	15
StainTemp	Stain Temperature (°C)	15	50	25
FirstStainTime	First Stain Time (seconds)	0	86399	600
CWashTemp	Wash A2 Temperature (°C)	15	50	25
CWashCycles	Number of Wash A2 Cycles	0	99	10
CWashMixes	Mixes per Wash A2 Cycle	1	99	4
SecondStainTime	Second Stain Time (seconds)	0	86399	600
ThirdStainTime	Third Stain Time (seconds)	0	86399	600
DWashTemp	Wash A3 Temperature (°C)	15	50	30
DWashCycles	Number of Wash A3 Cycles	0	99	15
DWashMixes	Mixes per Wash A3 Cycle	1	99	4
HoldTemp	Holding Temperature (°C)	15	50	25

The following are parameters for a 2-wash staining (Table E.2).

Table E.2
Parameters for a 2-washing staining

Parameter Name	Description	Parameter Values		Default
VAR: HybTime	Hybridization Time (seconds)	0	86399	1800
VAR: HybTemp	Hybridization Temperature (°C)	15	50	45
VAR: FlushTemp1	Flush A Temperature (°C)	15	50	30
VAR: FlushCycles1	Number of Flush A Cycles (2ml)	0	99	5
VAR: AWashTemp	Wash A Temperature (°C)	15	50	30
VAR: AWashCycles	Number of Wash A Cycles	0	99	4
VAR: AWashMixes	Mixes per Wash A Cycle	1	99	10
VAR: FlushTemp2	Flush B Temperature (°C)	15	50	30
VAR: FlushCycles2	Number of Flush B Cycles (2ml)	0	99	5
VAR: BWashTemp	Wash B Temperature (°C)	15	50	30
VAR: BWashCycles	Number of Wash B Cycles	0	99	1
VAR: BWashMixes	Mixes per Wash B Cycle	1	99	1
VAR: FlushTemp3	Flush B Temperature (°C)	15	50	30
VAR: FlushCycles3	Number of Flush B Cycles (2ml)	0	99	0

LCD Messages

The following is a list of some of the common messages that appear on the fluidics station LCD throughout the course of the fluidics station operation. The actual list of messages on a particular fluidics station may include some that are not listed here (Table E.3).

Table E.3
LCD Messages

Message	Meaning
Changing –>	Wait for temperature to reach set point
Draining to Waste	Empties cartridge.

Table E.3
LCD Messages

Message	Meaning
Purging with A	Purges chip with ~ 2ml of buffer A at 25°C from bottom to top then to waste.
Draining to Vial 1	Recovers stain to Vial #1 for reuse or disposal.
Draining to Vial 2	Recovers stain to Vial #2 for reuse or disposal.
Draining to Vial 3	Recovers stain to Vial #3 for reuse or disposal.
Filling with A or Filling with B	Drains and fills cartridge with last wash solution used, if any.
EJECT WASHBLOCK	Prompt to disengage washblock.
LOAD CARTRIDGE	Prompt to loading cartridge.
REMOVE PREVIOUS VIALS	Prompt to remove vials.
LOAD VIALS 1-2-3	Prompt for loading vials 1, 2, and 3.
LOAD VIALS 1& 2	Prompt for loading vials 1 and 2.
LOAD VIALS 1& 3	Prompt for loading vials 1 and 3.
LOAD VIAL 1	Prompt for loading vial 1.
LOAD VIAL 2	Prompt for loading vial 2.
LOAD VIAL 3	Prompt for loading vial 3.
Filling with A	Empty, fill with wash-A, mix by drain-and-fill, repeat, leave cell full.
Filling with B	Empty, fill with wash-B, mix by drain-and-fill, repeat, leave cell full.
Draw 1st Stain	Empty, draw sample to both sensors.
Draw 2nd Stain	Empty, draw sample to both sensors.
Draw 3rd Stain	Empty, draw sample to both sensors.
EJECT CARTRIDGE	Prompt for removal of cartridge.
ENGAGE WASHBLOCK	Prompt for engagement of washblock.
DO CLEAN CYCLE	Begin cleaning cycle.
REMOVE STAIN VIALS	Prompt to remove vials, if present.

Table E.3
LCD Messages

Message	Meaning
LOAD 3 EMPTY VIALS	Prompt to load vials.
Purging with water	Purge with 5ml water to clean line.
Washing needle 1	Performing wash needle #1 procedure.
Purging with air	Purge with air.
Washing needle 2	Performing wash needle #2 procedure.
Washing needle 3	Performing wash needle #3 procedure.
Washing Lines	Wash tube from valve to waste.
Prime Lines	Equilibrate tube from valve to waste with wash A.
REMOVE ALL VIALS	Prompt to remove vials.
LOAD SAMPLE VIAL IN LOC 1	Prompt for loading sample vial in location 1.
Flushing with WashA	Flushing with wash solution A.
Filling with WashA	Empty, fill with wash-A, mix by drain-and-fill, repeat, leave cell full.
A washes D/F	Wash with A by mixing using drain-and-fill procedure.
Flushing with Wasp	Flushing with wash solution B.
B washes D/F	Wash with B by mixing using drain-and-fill procedure.
REMOVE SAMPLE VIAL	Make sure sample vial is removed.
LOAD EMPTY VIAL IN LOC 1	Make sure empty vial is present.
Flushing with Wasp	Flushing with wash solution B.
Draining to Waste	Drain waste.
needle 1 w/Wasp	Flush needle 1 with Wash B.
Washing needle	Wash needle with water.
REMOVE VIAL	Prompt to remove vial.
Washing done	Completion of washing.
READY	System is ready.

Index

Index

Index

R

S

T

W