GENOMIC SOLUTIONS®

HybStation User Manual

GeneTAC® HybStation

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Section 1

Introduction to this User Manual

Contents

Warranty Statement

The warranty period for this equipment is as defined in the quotation upon which the order for the equipment was placed. The validity of the warranty is conditional upon the product being used strictly in accordance with the information provided in this User Manual.

No liability is accepted for loss or damage, or consequential loss or damage arising from incorrect use of the equipment. The liability of the Company within the guarantee period is limited to repair or replacement of the unit and, at the sole discretion of the Company, to the refund of the purchase price.

The HybStation is a complex piece of laboratory equipment. Although the equipment and software have been designed for ease of use, it is important to read the User Manual carefully. If in doubt about any procedures, contact Genomic Solutions.
Only trained personnel should operate the equipment.

Record of Revisions

Version	Date of issue	Amendments made
4.0	February 2003	Issued to Software Version 3.61

Safe Working Practices

Before operating the HybStation or any associated equipment, this User Manual must be read carefully, particularly observing all the safety warnings and precautions as stated.

During normal use, the HybStation will not present an electrical hazard to the operator, but **situations could arise where hazardous conditions may be present**.

The equipment is intended for use by qualified personnel who recognize electrical shock hazards and are familiar with the safety precautions required to avoid possible injury.

To maintain a safe working environment and protect user personnel at all times, the following safe working practices must be adhered to when using the equipment:

- **Maintain a well-ventilated laboratory**.
- **Always keep the work area dry**. Always work with dry hands and ensure that the work area is kept dry during use.
- **Maintain proper clearance on underside and sides of the instrument for proper air conduction**.
- **Ensure that the power outlet is properly grounded (earthed)**. Each time the equipment is used, the integrity of the main power cable must be inspected.
- **Be aware that high voltages are an electrical shock hazard**. For optimum safety, do not touch the power cable while power is applied. Always switch OFF the power at the mains supply socket before connecting or disconnecting any cables or equipment.
- **Replacements for blown fuses must be of the same type and rating as the original**. Using an incorrect fuse could result in a fire hazard.

It is also essential that the HybStation be stored and operated within in a properly maintained laboratory environment to achieve optimum long-term performance.
The environment must be dust and vibration free and be controlled to achieve:

- Temperature between 5^{o}C and 40^{o}C
- Maximum relative humidity of 80% for temperatures up to 31^{o}C, decreasing linearly to 50% relative humidity at 40^{o}C.
- Pollution degree 2 environment in accordance with IEC 664.

Power Connection

The equipment is supplied with a separate main power lead having an integral ground (earth) wire. The main power lead must firstly be inserted into the power socket at the side of the workstation, as shown above. The main outlet plug is then inserted into a properly grounded power supply socket.

If any doubt exists about the power supply socket intended for use, a qualified electrician must be contacted for advice.
It must be noted that the workstation is equipped with a universal power supply. However, the controlling PC is not equipped with a universal power supply, so the PC voltage is factory set prior to dispatch from Genomic Solutions. The PC supply voltage setting must therefore not be adjusted, except by Genomic Solutions personnel.

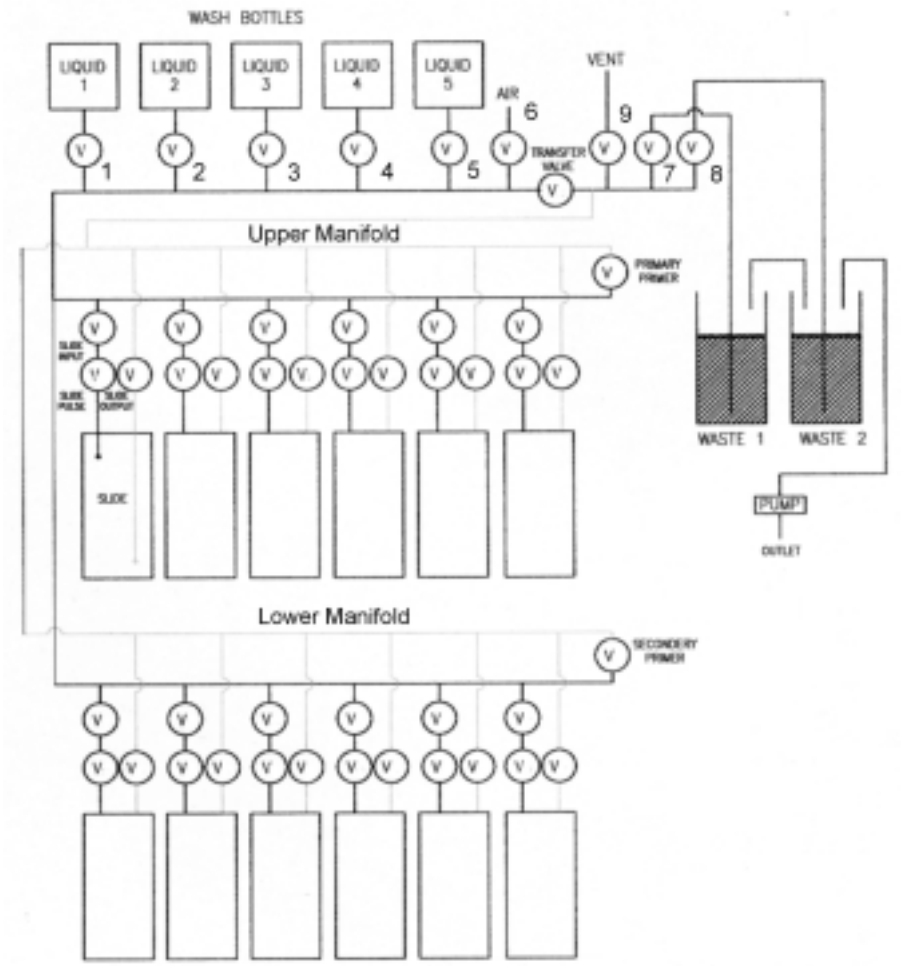
Warning: *Failure to provide a properly grounded power socket for the equipment could lead to an electrical shock hazard.*

It is also important to ensure that main supply voltage fluctuations do not exceed +/- 10% of the supply voltage.

Main Power Lead

Section 2

The GeneTAC HybStation



The HybStation System

Introduction

The GeneTAC HybStation is an automated system for hybridizing and washing microarrays. Protocols can be run simultaneously to achieve uniform reproducible results with minimal handling of solutions and slides.

Each microarray has a port for independent addition of labeled sample. The closed environment minimizes evaporation.

System Overview

The HybStation has six Peltier temperature-controlled devices with fluidics capabilities.

For each module, two microscope slides are placed (array element side facing upwards) on the thermally controlled Peltier unit. A polysulphone slide cover with chemically inert neoprene O-rings and a stainless steel shim is placed over the slides to create the hybridization chamber. Fluids enter the hybridization chamber through channels in the slide cover and are controlled by valves mounted inside an acrylic manifold. A vacuum source moves fluids through the system to the waste bottles.

Temperature control and fluidics can be varied on the HybStation to control hybridization of microarrays.

Temperature control has a range between 1 and 99° C (± 0.5° C) with temperature ramping of 1° C per second.

By separating the slide from the slide cover with a shim of a specific thickness, it is possible to maintain a consistent hybridization volume over time, regardless of the changing properties of the O-ring.

System Flowpath

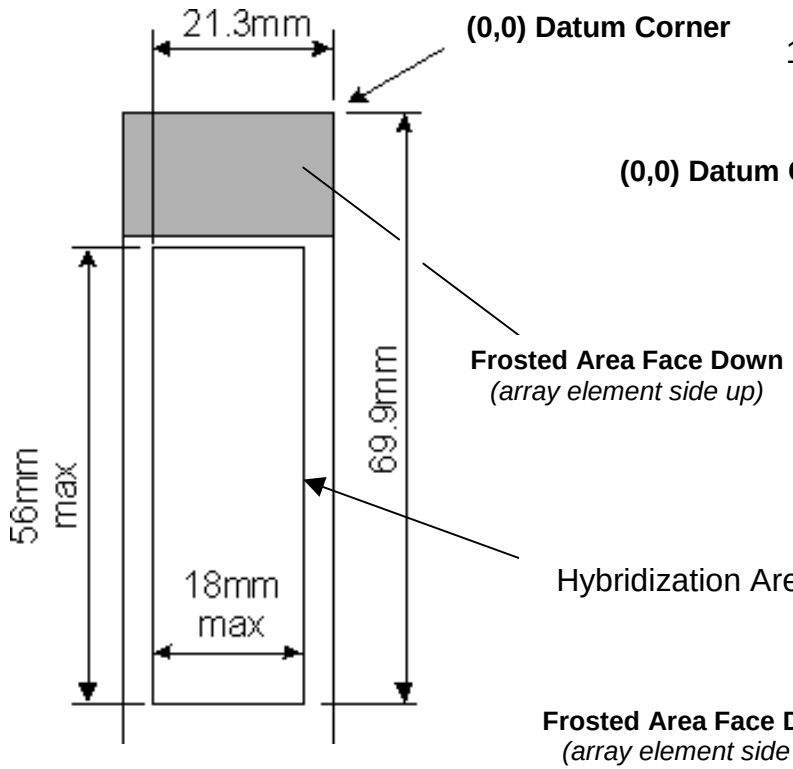
The flow path of the acrylic manifolds, valves, and tubing lines is shown on the page above:

On the diagram the reagent lines are numbered #1, #2, #3, #4, and #5.

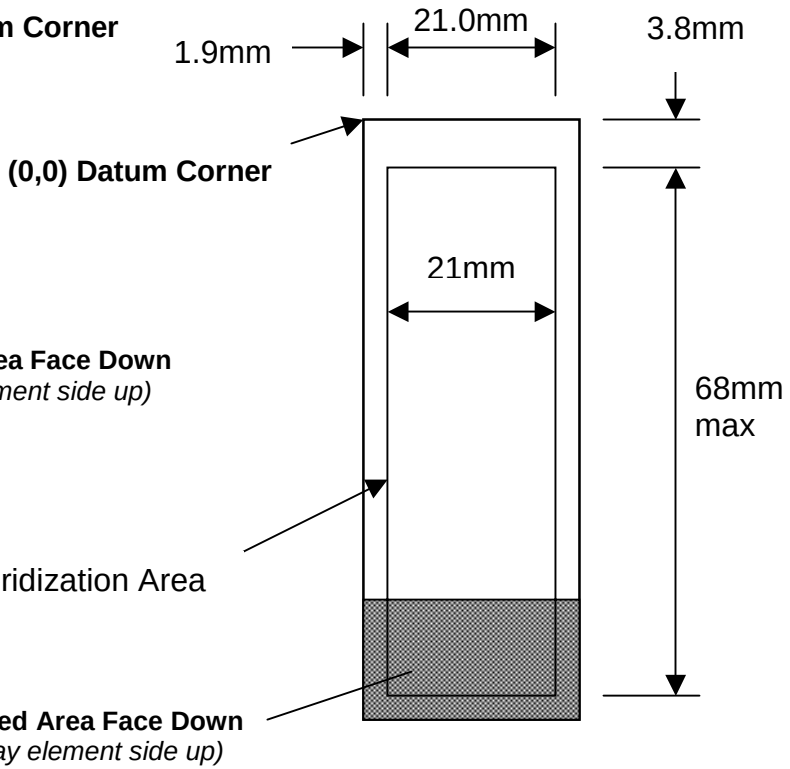
The four lines at the back of the manifold are #6 which is open to air, #7 for waste bottle #1, #8 for waste bottle #2, and #9 which is a vent line open to air.

There are three valves for each slide:

- Inlet valve
- Agitation valve: located between the inlet valve and the slide chamber
- Outlet valve



The 18mm Slide Assembly



The 21mm Slide Assembly

Introduction to Using Slides

The GeneTAC HybStation processes up to 12 slides on six temperature-controlled modules by washing fluids over the active surface of each slide. It is important to always place and seat slides properly on the instrument.

Slide Sizes

Two types of slide array assembly can be used with the HybStation; an 18mm type slide assembly and a 21mm assembly. The 18mm type slide assembly has a hybridization area of 18mm x 56mm, whereas the 21mm assembly provides a larger hybridization area of 21mm x 68mm, as shown in the figure above.

It must be noted that the two assemblies are quite different and their components are therefore not interchangeable.

An upgrading kit is now available to enable use of the 21mm assembly on any HybStation.

Frosted Slides

The frosted side of a slide **must always** be placed face-down on the carrier**.**
It is essential that hybridization is always carried out on the unfrosted side of a slide.

The Slide Sealing System

To retain the fluids during use, a polysulphone cover is fitted over the active surface of the slide. A gasket (neoprene O-ring) is integrated in the cover to seal the slide. It is critical that the gasket does not overlap the spots on the slide.

Critical Handling Notes

When handling slides, follow these recommendations:

- Do not touch or label the active surface of the slide. Fingerprints, labels, and pen marks may leach contaminants or prevent sealing by the O-rings.
- Apply labels to the inactive face of the slide at the end nearest the home position corner. Maximum rear face irregularity is +/- 50µm, that is, maximum label thickness is 100µm.
- Process slides in pairs. If it is required to process an odd number of slides, use a dummy (blank) slide in the unused position. This should be of the same slide type as the one being hybridized.
- Take care to never mark or score the glass slides in any way.

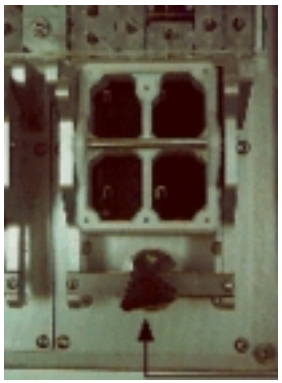
Using the 18mm Slide Assembly

A slide carrier is placed under each pair of slides to set the position. The home position is (0,0).

Placing Slides

To place slides on the GeneTAC HybStation:

1. Unscrew the black knob that holds the slide cover in position. This releases the cover from the thermal module assembly.

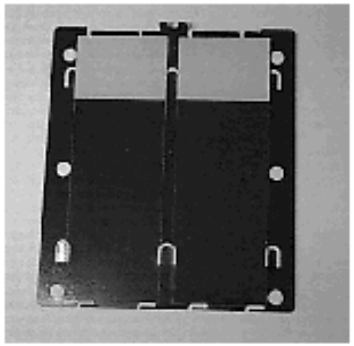


Black Securing Knob

2. To remove the slide cover, gently pull it forward and up.

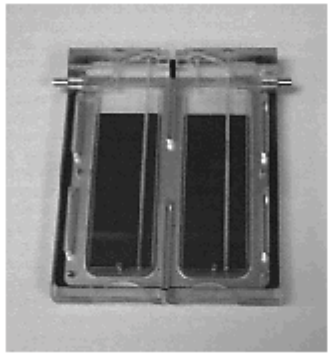
The top of the slide cover contains four small red connecting O-rings that seal against the reagent manifold. There is an indented area at the top center that encloses a small metal guide post that allows the cover to be repositioned.

3. Check the O-rings to make sure they are positioned correctly within the slide cover.
4. Place the slides one at a time onto the metal slide holder. The metal slide holder is flanged at the top, bottom, and sides to hold each slide in position. For correct placement, ensure that the slide is positioned tightly against the top right-hand edge of the flanged carrier plate with the array elements facing upwards.

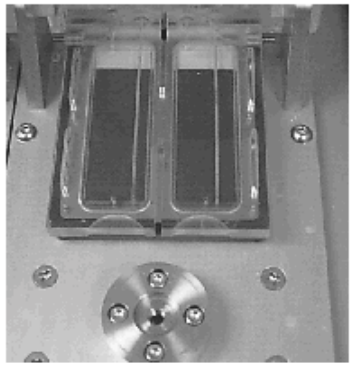


5. Place the slide cover into position over the slides and the flanged carrier plate. Ensure that the indentation in the top of the block is resting against the flanged edge at the top of the metal slide holder.

6. Gently place the slide carrier/cover assembly onto the module, positioning the block assembly against the metal guide pin.



7. Lower the clamp mechanism into position over the slide cover. To ensure a good seal, make sure that the slides do not move when positioning the clamp on top of the block.



Note: *If the system is sealed properly, the four red O-rings appear as fat round circles when viewed through the back of the manifold.*

8. Re-attach the locking mechanism by turning the black knob screw assembly until fingertip tight.

   Note: *Do not over-tighten the knob*

9. Continue placing slides on each module as necessary, following steps 1 through 8 until all slides are positioned for the run.

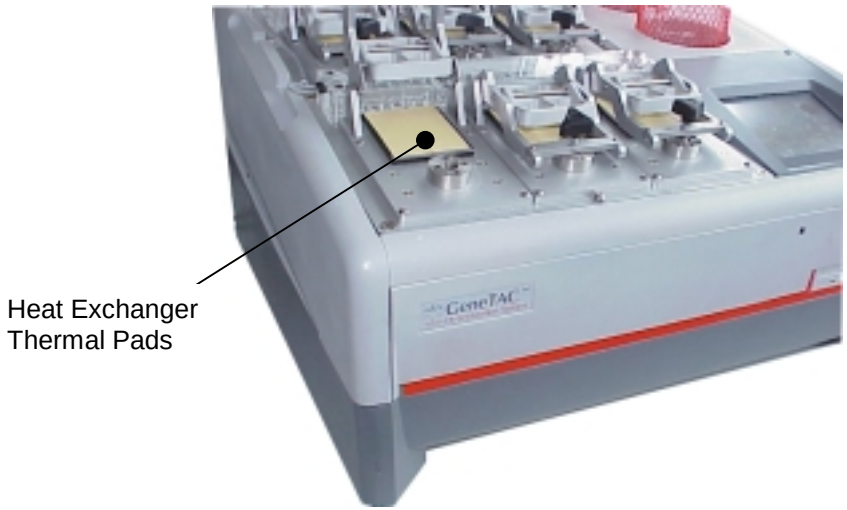
Using the 21mm Slide Assembly

The 21mm slide assembly provides a larger hybridization area than the 18mm assembly.

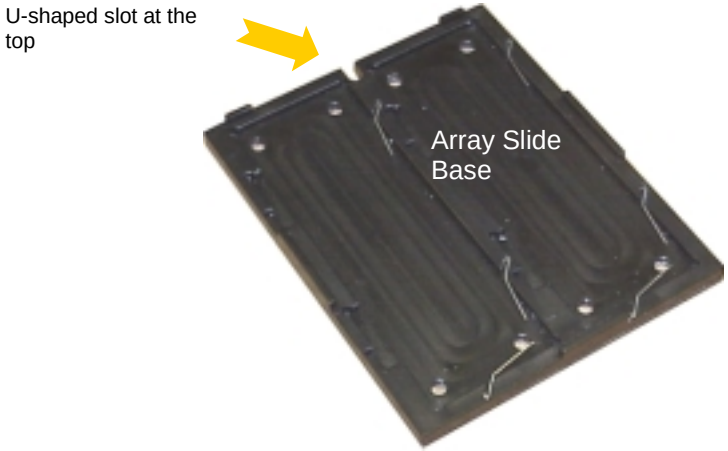
Note: *The 21mm assembly is clamped onto the HybStation in a similar way to the 18mm slides, so those details are not repeated again in this section.*

When in use, the complete slide assembly is located onto the (yellow colored) heat exchanger thermal pads positioned on the HybStation heat exchangers. It must be noted that these Thermal Pads should always be left in position on the heat exchangers and must not be removed.

To use the 21mm assembly, proceed as follows:



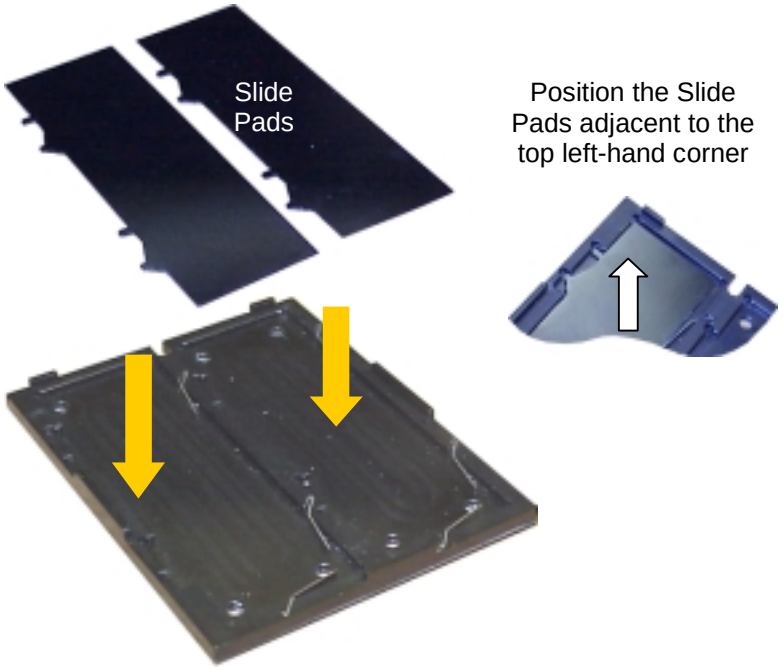
Note: *When setting up and using the 21mm slide assembly, always ensure that the U-shaped slot is located at the top of the Array Slide Base*



To use the assembly, proceed as follows:

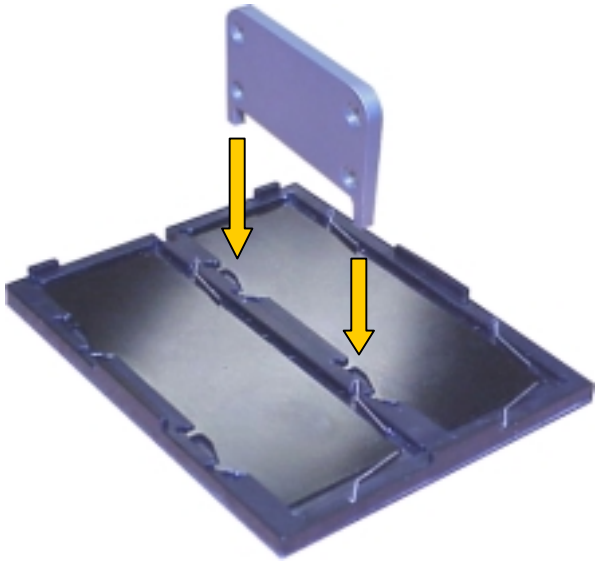
1. Place two Slide Pads into the recesses of the Slide Base.

When positioning the pads, care must be taken to ensure that they are pushed towards the top left-hand corner of the recesses and that the springs are properly positioned flush with the base.
These pads will later be used to compress the springs to allow insertion of the Array Slides.



2. With the slide pads correctly positioned, place the insertion tool into the recesses on the left-hand side of one of the slide pads.

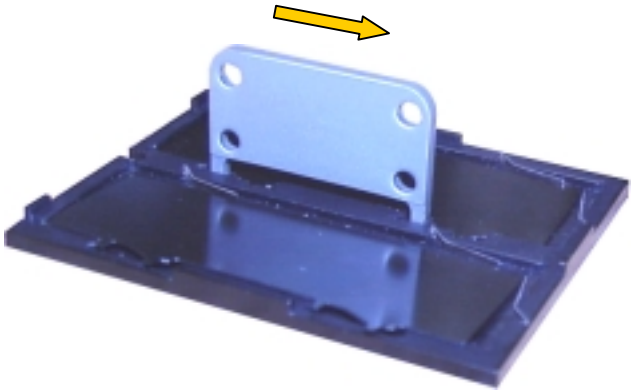
This tool will then be used to push the slide pads towards the bottom right-hand corner of the base, against the action of the springs.



3. Holding the Insertion Tool in position slide the tool towards the bottom of the Array Slide Base until it clicks into the insertion position.

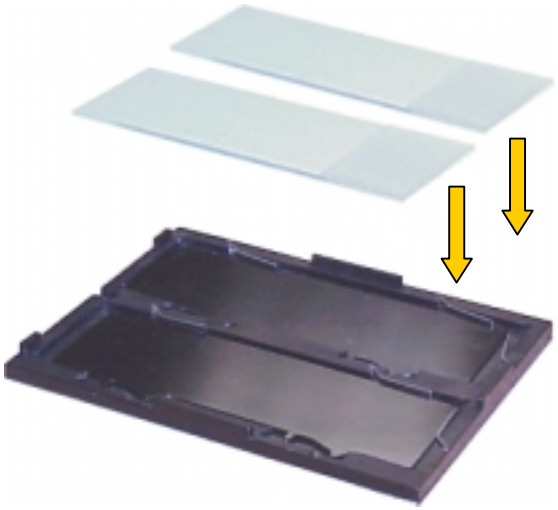
As the tool slides along, the Slide Pad compresses the springs until the pad clicks into position. When the tool has clicked into this position, the Slide Pad will remain fixed and the tool can be withdrawn.

***Note:** It is important to ensure that the springs remain properly seated and that each of the Slide Pads are secure.*



4. With the springs compressed, the two Array Slides can be dropped into the recesses of the Array Slide Base.

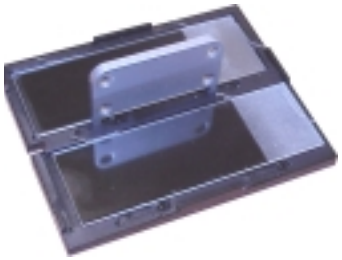
Ensure that the slides are properly seated and that the white / frosted area of each slide is positioned at the bottom of the base, facing downwards. It is also important to ensure once again that the springs are properly seated flush against the base and the edges of the Slide Pads.

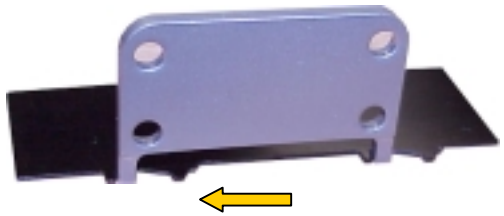


5. To properly position the slides, they need to be pushed towards the top left-hand corner of the Array Slide Base.

This corner acts as the system datum and the slides must always register with this datum corner when in use.

To move the slides into position, place the insertion tool into position as shown, then slide the tool towards the top of the assembly. This will move the Slide Pad and allow the springs to push the slides into the top left-hand datum corner.
When the slides are in position, check that they are properly seated against the datum corner and that the springs are properly positioned, flush with the base and against the edges of the slides.

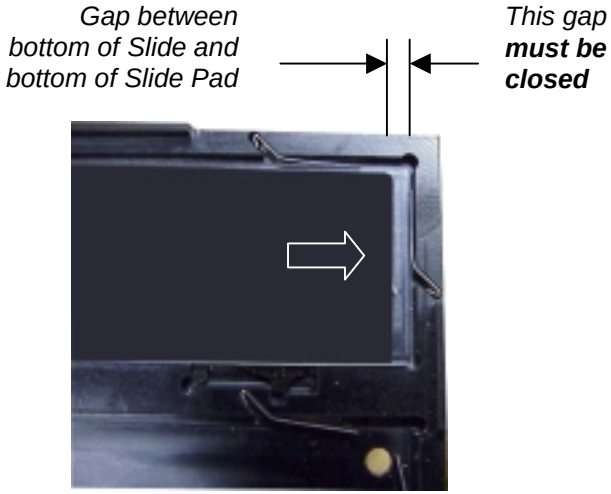




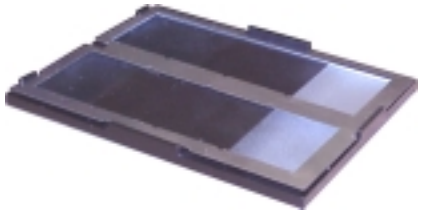
Push the Insertion Tool towards the top of the assembly, allowing the springs to position the slides at the datum corner

6. Using the Insertion Tool, carefully slide the Slide Pad downwards to bring the bottom of the Slide Pad level with the bottom of the Array Slide.

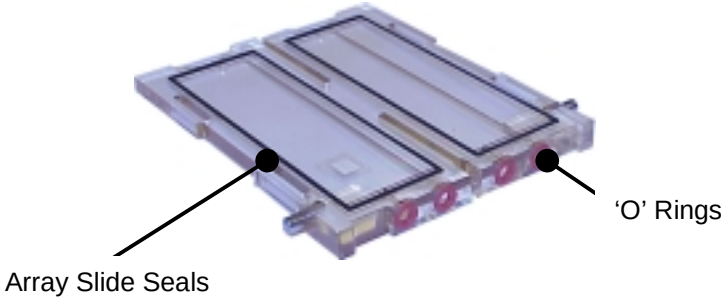
Note: *It is important that the edges of these are aligned and that no gap exists between them. Failure to do this could result in damage occurring to the Array Slides.*



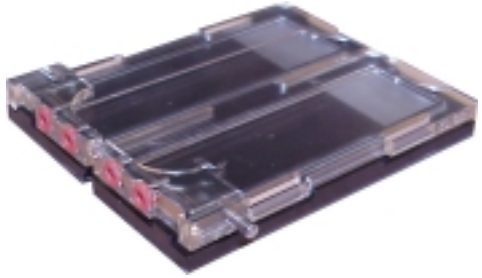
7. Place a metal shim onto the top of the assembly, as shown.



8. Prepare the Array Slide Cover for use. To do this, ensure that four red colored connecting ‘O’ Rings are correctly positioned into the recesses at the end of the cover, and insert two black Array Slide Seals into the rectangular recesses in the cover.



9. After finally checking that the Array Slides are correctly positioned, place the Array Slide Cover onto the assembly (with the ‘O’ Ring seals at the top).
The assembly is now ready for use.

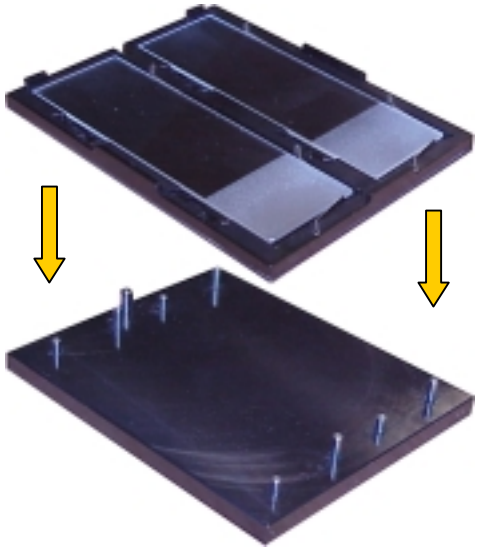


Removal of the Array Slides.

To remove the Array Slides from the assembly, first remove the Slide Cover. The Array Slide Base can then be positioned onto the Extraction Tool.

The pins protruding from this tool will release the Array Slides and Slide Pads when the base is pushed down onto it.

Remove the Array Slide Base from the Extraction Tool when the Array Slides and Slide Pads have been removed.



Note: *It must be noted that when removing the Array Slide Cover, the Array Slides may stick to the Array Slide Seals.*
This will depend upon the type of protocol being used and is quite normal. The slides can carefully be removed from the seals without any damage occurring.

Using Reagents

The GeneTAC HybStation has positions for five reagent or reservoir bottles and two waste bottles.

Note: Before starting any hybridization protocol it is important to empty the waste bottles and dispose of the contents properly.

Genomic Solutions Reagents

Genomic Solutions offers hybridization and wash buffer solutions for the GeneTAC HybStation. The GeneTAC Hybridization Kit for 12 to 15 hybridizations includes:

2 tubes of 2X HybBuffer Hybridization Solution (1ml each)

1 bottle GeneTAC Medium Stringency Wash Buffer (500ml)

1 bottle GeneTAC High Stringency Wash Buffer (500ml)

1 bottle GeneTAC PostWash Buffer (500ml)

1 package of neoprene O-rings

2 labeled sample port plugs

Your Genomic Solutions representative should be contacted for catalog numbers and pricing information.

Reagent Guidelines

Reagents provided by Genomic Solutions can be safely used with the GeneTAC HybStation. Use the wash solutions in order of increasing stringency.

Never use these solutions in the GeneTAC HybStation:

- All acids
- All bases
- Organic solvents, such as phenol, acetone, chloroform, xylene
- Chaotropic solutions, such as guanidine-HCl
- Ethanol

If a precipitate occurs, remove the damaged part and soak it in warm, ultrapure water overnight. If available, use a continuous flow waterbath, which will aid in dislodging the precipitate. Gentle abrasion with a soft brush may help to dislodge the precipitate from the slide cover, but take care not to scratch the part.

The manifolds are not removable.

The hybridization and wash solutions contain sodium dodecyl sulfate (SDS). This strong ionic detergent will precipitate out of solution when slightly chilled. Inspect solutions before use to assure that precipitation has not occurred. SDS will go back into solution upon warming.

Never use a solution that has a visible precipitate. Also, medium to high stringency buffers are always recommended for use.

Basic Maintenance

Cleaning the Case

Clean the exterior case of the GeneTAC HybStation with a mild detergent and rinse with distilled water.

Caution: *Risk of electrical shock can exist. Turn OFF power to the GeneTAC HybStation and disconnect the power cord before cleaning the instrument. DO NOT allow liquid to enter the inside of the power unit. Liquid may damage the power unit and present a potential shock hazard.*

Cleaning the Lines

After completing a hybridization, run a machine clean cycle wash using blank slides and ultrapure water. This step is particularly important if the machine will be idle for a few days.

Note: *Refer to Section 4.5, Cleaning the Valves and Lines.*

Maintaining the O-rings

Note: *It is recommended that the neoprene O-rings be after each run as worn O-rings will not seal properly and leaking could occur. If the O-rings are re-used, ensure that they are cleaned well before the next run is carried out*

To clean the O-rings:

- Remove the O-rings from the slide cover. Rinse the cover under a stream of water and gently lift an edge of the O-ring, or use canned air to remove the O-ring.
- Place the O-rings into a boiling bath of ultrapure water at approximately 80^{o}C for 1 to 2 minutes.
- Remove the O-rings from the bath and allow them to air dry.

To replace the O-rings:

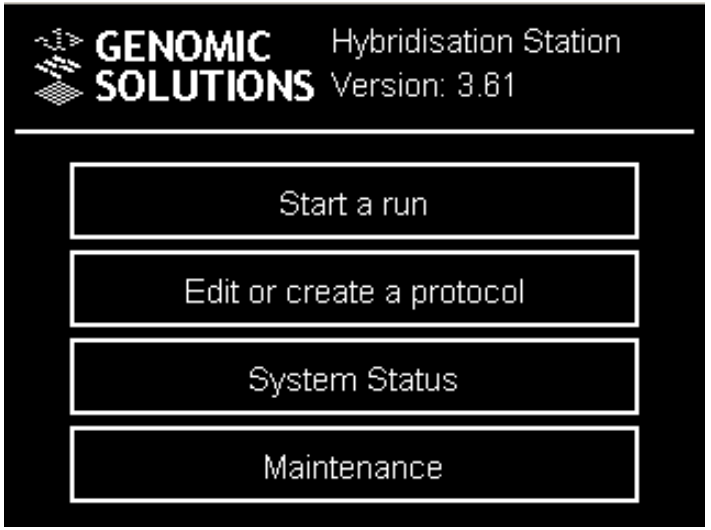
- Make sure the channel is free from particulate matter.
- Place the O-ring over the channel.
- Gently press the O-ring into the groove in the slide lid.
- Do not twist the O-ring when replacing it or the slide cover may not seal completely.

Section 3

Using the
HybStation

The ON / OFF Switch

The Main HybStation Screen



Initiating the GeneTAC HybStation

To initiate the GeneTAC HybStation, first ensure that there is no disc in the disc drive then place the ON / OFF switch into the ON position.

This switch is located at the back of the instrument.

The system will then start and the main control screen will open.


GENOMIC SOLUTIONS
Hybridisation Station
Version: 3.56
Start a run
Edit or create a protocol
System Status
Maintenance
GENOMIC SOLUTIONS
Select The Drive To Load From
Floppy Drive
Internal Drive
Cancel
GENOMIC SOLUTIONS
Choose A Protocol To Load
DEMO.HYB
PROBE.HYB
STD.HYB
TEST.HYB
Load
Delete
Cancel
Select slides for this run:
(Arrows indicate recommended slides)
1
2
3
4
5
6
7
8
9
10
11
12
Cancel
Select All
Start
Press 'Continue' when you are ready to start this run
Cancel
Continue

Running a Protocol

Starting a Run

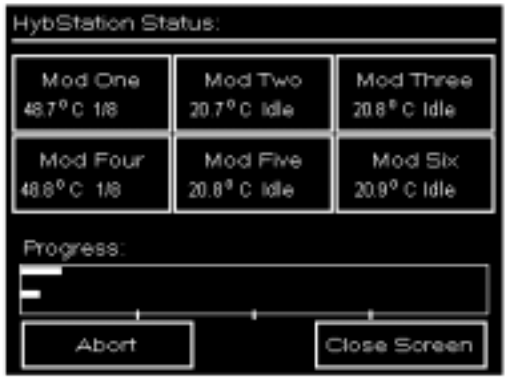
To run a protocol, proceed as follows:

1. At the Main control screen, select **Start a run**.
   The **Select The Drive to Load From** screen will then appear, prompting for the location of the protocol to be defined.
2. Select the location of the protocol (**Floppy Disk** *or* **Internal Storage)**. The **Choose a Protocol to Load** screen will then open, listing all available protocols.
3. Select the protocol to be run. A marker will appear next to the protocol selected.
4. Select **Load**. The **Select Slides for the Run** screen will then open. At this screen select the slides for use during the protocol. The selected slides will then become highlighted.

   Note: To deselect a slide, press the slide number again. To select all slides, press **Select All** at the bottom of the screen.
   The arrows indicate the slide positions selected. The system will select positions to ensure even use of all modules.
5. Select **Start**. A message screen will then open stating **Press Continue when you are ready to start this run.**
6. Place the slides into the slide holders.

   Note: *For details of how to place slides onto the HybStation, refer to Section 2.2*

When the slides have been correctly positioned press **Continue**. The **HybStation Status** screen will then open.



The use of this screen for monitoring purposes is described in Section 3.4, Monitoring a Run.

To exit this screen and return to the Main screen, select **Close Screen** .

Adding a Probe (Labeled Sample)

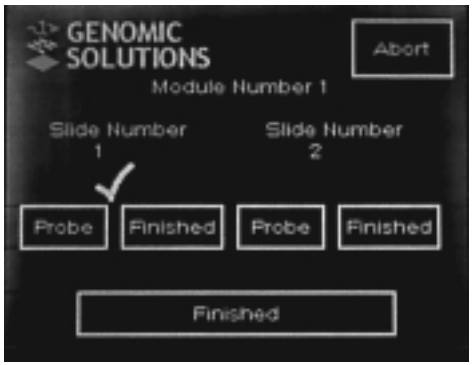
The HybStation software indicates when to add a labeled sample by displaying the prompt screen.

To add a labeled sample:

1. At the screen, press the **Probe** button to add a labeled sample for the slide. A check mark will then appear next to the **Probe** button.

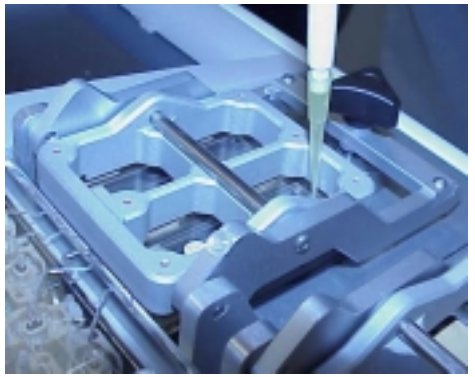
   Note: *A click will be heard as the valve opens to allow the system to vent. This valve must be opened to enable the labeled sample to enter the slide chamber.*

   In this example, the probe for slide number 1 is selected.



2. Inject the labeled sample by pipetting it slowly into the port using a standard 200ml pipette. If using the 18mm slide assembly it is recommended that 100µl to 110µl is used. This should be increased to approximately 140-150µl when using the 21mm slide assembly.

The probe port is located above a diffusion channel machined into the slide cover. There is also a complimentary diffusion channel at the top of the slide cavity. Capillary action tends to draw the labeled sample out of the first diffusion channel during insertion.



The labeled sample must fill up to the top diffusion channel. This area accommodates thermal expansion and contraction of the fluid and fluid displacement due to minor thermal expansion of the slide cover.

3. When the labeled sample has been added, insert the labeled sample plug.
4. Press the **Finished** button related to the slide where the labeled sample was added. The check mark on the screen will then disappear.
5. To add labeled sample to the second slide position, repeat steps 1 to 4.
6. When labeled sample has been added to the last slide, press **Finished** at the bottom of the screen. The system will then continue with the defined protocol.

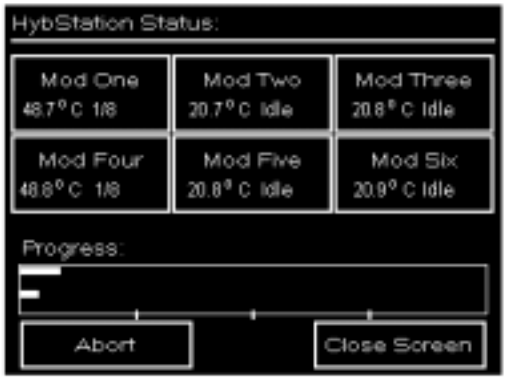
Monitoring a Run

A hybridization run is monitored at the **HybStation Status** screen. This screen identifies the modules and shows the current status:

- Module number
- Temperature
- Current step
- Total steps in the protocol

To monitor a run:

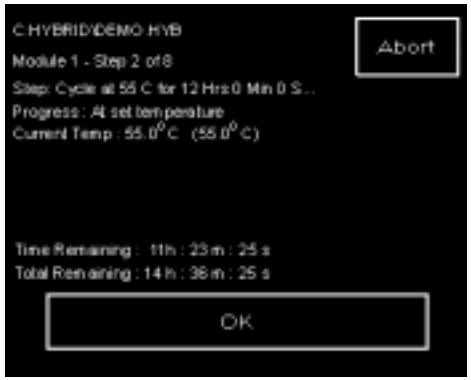
On the Main screen, press **System Status** . The **HybStation Status** screen will then open.



In this example, the *HybStation Status* screen shows the following:

- A protocol is running on Modules One and Four.
- The protocol has eight steps, and the first step is in progress.
- Modules Two, Three, Five, and Six are idle.
- The **Progress** area shows a progress bar for each active module. *Modules One through Six correspond to six progress bars from top to bottom*.

To view details of a specific module, press the box representing the module. A screen will then open showing detailed information.



This example shows the following information:

- The name of the protocol on this module is DEMO.HYB.
- This is Module One.
- The second step of an eight-step protocol is currently running.
- The current step is to cycle at 55° C for 12 hours.
- The module is at the set temperature for the current step.
- The current temperature is 55° C, *the number in parentheses indicates the array element temperature.*
- Time Remaining refers to the time remaining for the current step.
- Total Remaining refers to the time remaining for the current protocol.

To return to the **HybStation Status** screen, press **OK** .

To return to the Main screen, press **Close Screen** on the **HybStation Status** screen.

Aborting a Run

If necessary, a run can be aborted on all modules or on selected modules.
To abort the run on all modules:

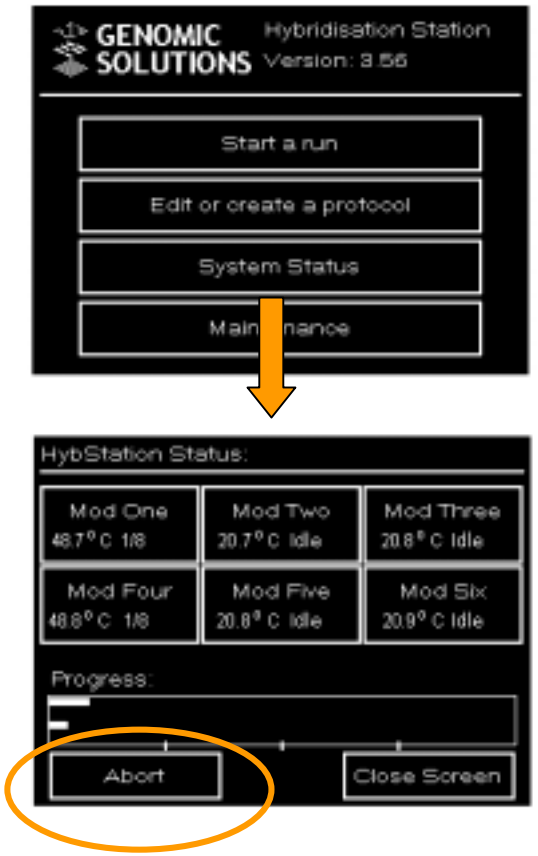
1. On the Main screen, press **System Status** . The **HybStation Status** screen appears.
2. Press **Abort** . A Confirmation screen will then appear. Press **Yes** . The Main screen appears.

To abort the run on selected modules:

1. On the Main screen, press **System Status** . The **HybStation Status** screen appears.
2. Select the module on which the run is to be aborted. The **Status** screen for the module will appear.
3. At the upper right-hand area of the screen, press **Abort** . A Confirmation screen will then appear.
4. Press **Yes**. The **HybStation Status** screen will then appear.

To abort the run on more modules, repeat steps 2 and 3 above.

To return to the Main screen, press **Close Screen**.



Creating a Protocol

A new protocol can be created using an external PC and then be run directly (or saved on a floppy disk), or can be created on the HybStation embedded PC for repeated use.

The procedure in this section describes in general how to create a protocol.

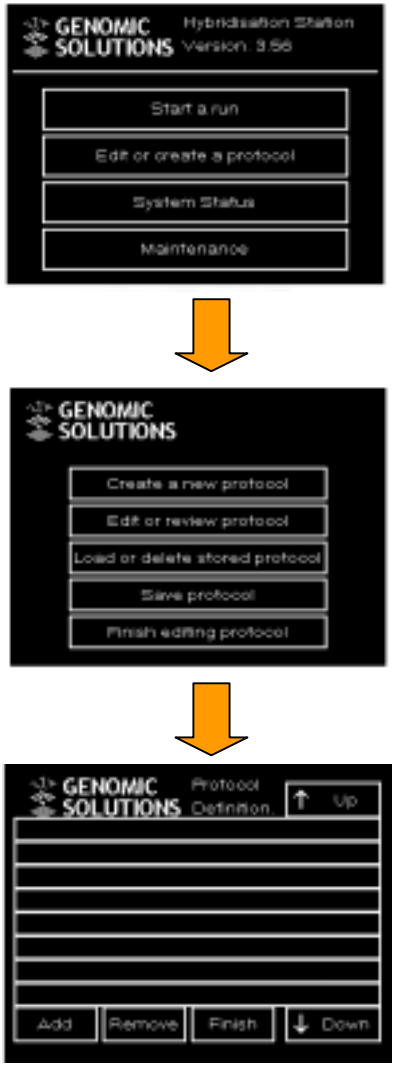
To create a new protocol:

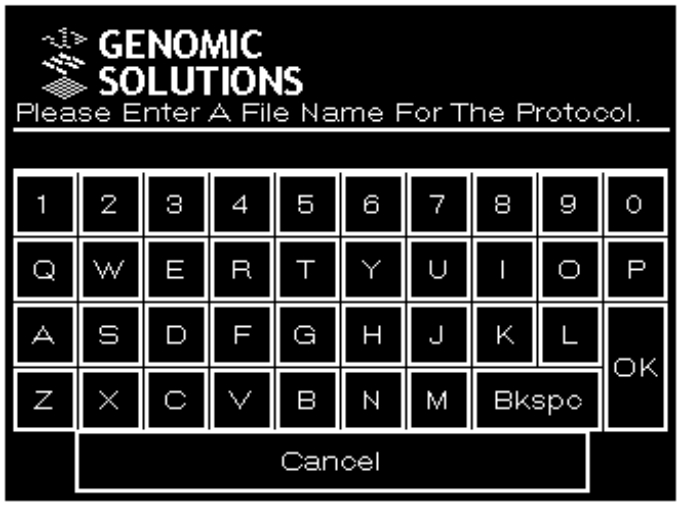
1. On the Main screen, press **Edit or create a protocol** . A new screen will then appear.
2. Press **Create a new protocol** . The **Protocol Definition** screen will then appear.
3. To begin adding steps, press **Add**. The **Steps** screen will then appear.

   When a step has been added, the system will return to the ***Protocol Definition*** screen.
4. Continue adding as many steps as necessary for the protocol.
5. Review the protocol steps; use the **Up** and **Down** arrows to scroll through the steps.

   Note: *To remove one of the steps, select the step and press the* **Remove** *button.*
6. To finish the protocol, press the **Finish** button.

   Note: *The top right-hand area of the screen will indicate that a protocol has been defined.*
7. Press **Save Protocol**. The Keypad screen will then appear.




GENOMIC
SOLUTIONS
Please Enter A File Name For The Protocol.
1 2 3 4 5 6 7 8 9 0
Q W E R T Y U I O P
A S D F G H J K L
OK
Z X C V B N M Bkspc
Cancel


You have not saved this protocol.
Are you sure you want to lose the
changes you have made?
Yes
No

8. At the **Please Enter a File Name for the Protocol** screen, enter a name for the protocol using up to eight alphanumeric characters. To do this use the alphanumeric buttons, noting the following:

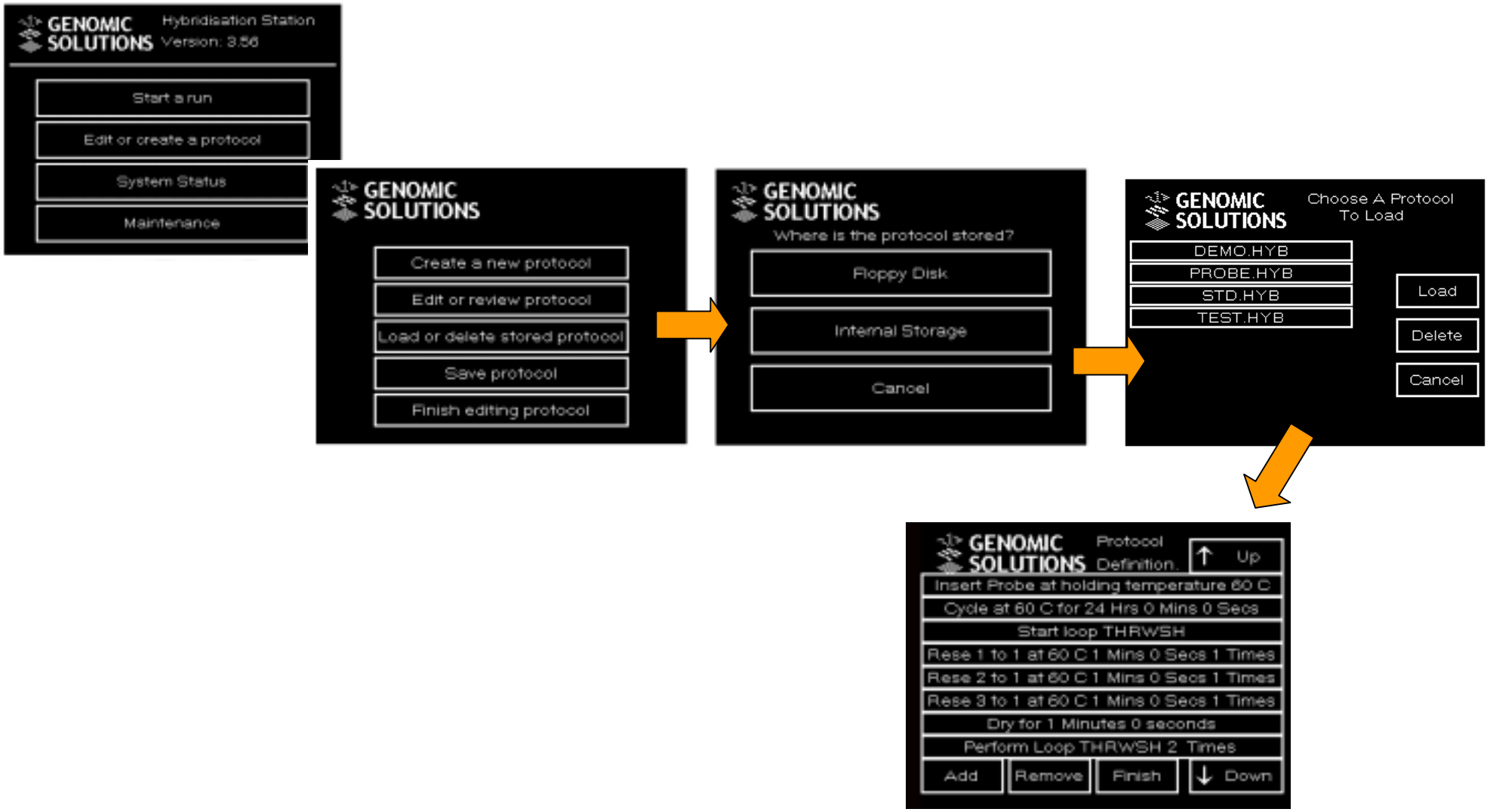
- To delete a character, press Bkspc.
- To cancel the operation and return to the *Protocol* screen, press **Cancel**.

To accept name the entry and continue, press **OK**. The Main screen will then appear.

If **Finish** on the **Protocol Definition** screen is pressed before the new protocol is saved, a warning screen will appear stating:

You have not saved this protocol. Are you sure you want to lose the changes you have made?

To save the protocol, press **No** .The **Protocol** screen then appears. Press **Save Protocol** and proceed as described above.


GENOMIC SOLUTIONS
Hybridisation Station
Version: 3.56
Start a run
Edit or create a protocol
System Status
Maintenance
GENOMIC SOLUTIONS
Create a new protocol
Edit or review protocol
Load or delete stored protocol
Save protocol
Finish editing protocol
GENOMIC SOLUTIONS
Where is the protocol stored?
Floppy Disk
Internal Storage
Cancel
GENOMIC SOLUTIONS
Choose A Protocol To Load
DEMO.HYB
PROBE.HYB
STD.HYB
TEST.HYB
Load
Delete
Cancel
GENOMIC SOLUTIONS
Protocol Definition
Up
Insert Probe at holding temperature 60 C
Cycle at 60 C for 24 Hrs 0 Mins 0 Secs
Start loop THRWSH
Rese 1 to 1 at 60 C 1 Mins 0 Secs 1 Times
Rese 2 to 1 at 60 C 1 Mins 0 Secs 1 Times
Rese 3 to 1 at 60 C 1 Mins 0 Secs 1 Times
Dry for 1 Minutes 0 seconds
Perform Loop THRWSH 2 Times
Add
Remove
Finish
Down

Editing an Existing Protocol

An existing protocol can be edited and saved under the same or a new name for repeated use. Editing a protocol effectively means adding or removing steps.

Note: *It is only possible to edit a protocol that was created with HybStation software. This is done using the touch screen.*

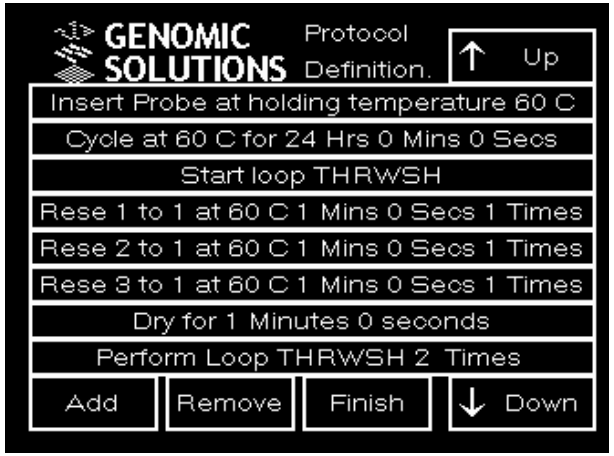
Selecting a Protocol for Editing

A protocol must be loaded before it can be edited.

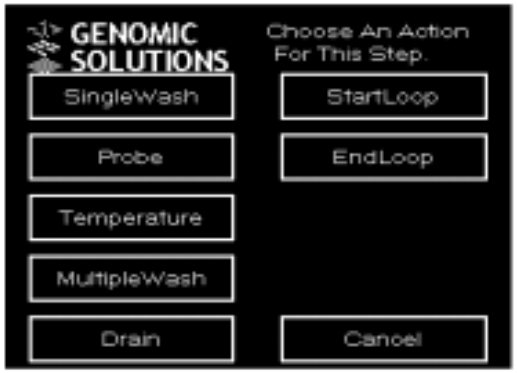
To select a protocol for editing, proceed as follows:

1. On the Main screen, press **Edit or Create a Protocol** . The Protocol screen will then appear.
2. On the Protocol screen, press **Load or Delete Stored Protocol** . A screen appears, prompting **Where is the Protocol Stored?**
3. Select **Floppy Disk** or **Internal Storage** . A list with available protocols will appear.
4. From the list, select the protocol to be edited. A marker to the right-hand of the protocol name indicates the selection.
5. Press **Load**. The Protocol screen appears, indicating at the top right-hand area that a protocol is defined.

On the Protocol screen, press **Edit or review protocol**. The **Protocol Definition** screen appears, listing the steps in the protocol. This can then be edited as described on the following pages.



A Typical Protocol Definition



Adding Steps to a Protocol

1. Select a protocol for editing as described on the previous page.
2. Add a new step either before a specified step or at the end of a protocol:
   - ❑ To add a step before a specified step, select the step on the **Protocol Definition** screen. A marker will appear to the right of the step. Then, press **Add** at the bottom of the screen.
   - ❑ To add a step to the end of the protocol, press **Add** at the bottom of the screen without selecting a step.

   To return to the Protocol screen without adding steps, press **Finish**.
3. Press the step to be added.

   Note: *For a description of all available steps, refer to Section 3.11*
4. Repeat steps 2 and 3 to add more steps to your protocol.
5. When all required steps have been added, press **Finish** . The Protocol screen will then appear.
6. On the Protocol screen, press **Save protocol** . The Keypad screen will then appear.
7. Enter a name for the protocol of up to eight alphanumeric characters using the alphanumeric buttons, noting the following:
   - ❑ To delete a character, press Bkspc .
   - ❑ To cancel the operation and return to the *Protocol* screen, press **Cancel** .
   - ❑ To accept your entry and continue, press **OK** . The Main screen will then appear.

   If **Finish** on the **Protocol Definition** screen is pressed before the new protocol is saved, a warning screen will appear stating:

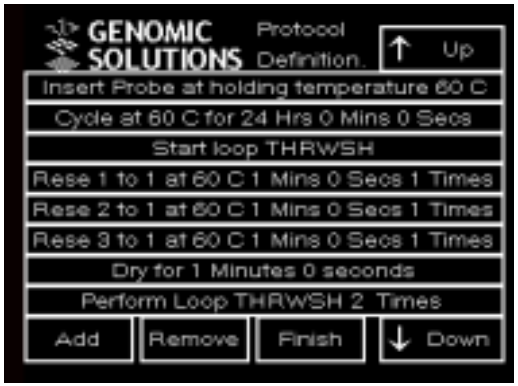
   You have not saved this protocol. Are you sure you want to lose the changes you have made?

   To save the protocol, press **No** .The **Protocol** screen then appears. Press **Save Protocol** and proceed as described above.

Removing Steps from a Protocol

To remove steps from a protocol:

1. Select a protocol for editing as described on the previous pages.
2. Select the step to be removed. *A marker to the right of the step indicates the selection made.*



3. Press **Remove**. The program immediately removes the step from the protocol.
4. Remove additional steps by repeating the above, as required.
5. When the required steps have been removed, press **Finish** . The Protocol screen will then appear. On the Protocol screen, press **Save Protocol** . The Keypad screen will then appear.

6. Enter a name for the protocol of up to eight alphanumeric characters using the alphanumeric buttons, noting the following:
   - To delete a character, press Bkspc .
   - To cancel the operation and return to the Protocol screen, press **Cancel.**
   - To accept your entry and continue, press **OK**. The Main screen will then appear.
   -

If **Finish** on the **Protocol Definition** screen is pressed before the new protocol is saved, a warning screen will appear stating:

You have not saved this protocol. Are you sure you want to lose the changes you have made?

To save the protocol, press **No** .The **Protocol** screen then appears. Press **Save Protocol** and proceed as described above.

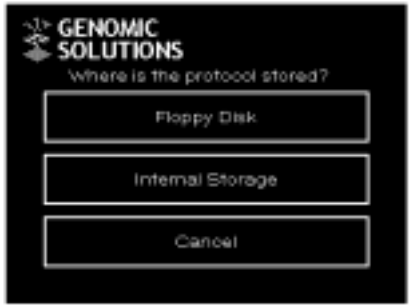
Deleting a Protocol

A protocol can be deleted from either a floppy disk or the internal PC storage.

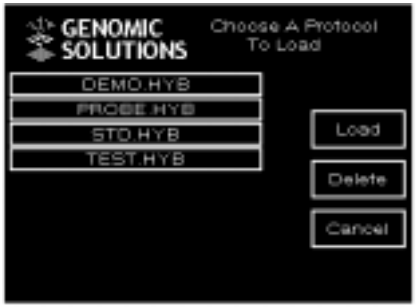
To delete a protocol:

1. On the Main screen, press **Edit or create a protocol** . The Protocol screen will then appear.

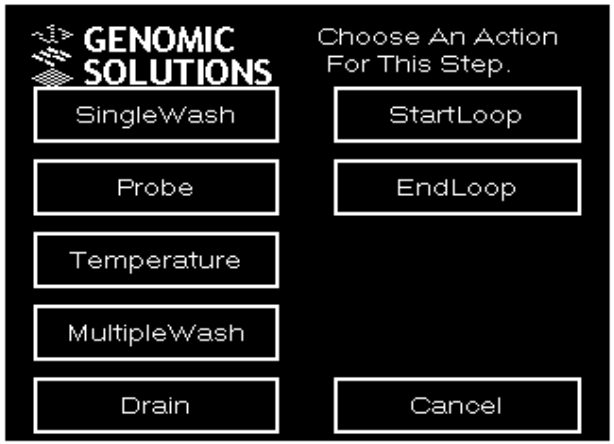
2. On the Protocol screen, press **Load or delete stored protocol** . A screen then appears requesting the location of the protocol to be deleted.



3. Press **Floppy Disk** or **Internal storage** . A screen then appears, listing all available protocols.



4. Select the protocol to be deleted. *A marker to the right of the protocol will indicate the selection.*
5. Press **Delete** . The system then removes the protocol.


GENOMIC SOLUTIONS
Choose An Action For This Step.
SingleWash
Probe
Temperature
MultipleWash
Drain
StartLoop
EndLoop
Cancel

The Protocol Steps

When steps are added to a new protocol or to a saved protocol, these steps can consist of the following, (and as shown in the above window).

All steps are initiated using the above screen.

- **Single wash** Select a solution to flow through the slide chambers.
- **Probe** Specify where in the protocol the labeled sample is to be added and at what temperature.
- **Temperature** Enter the temperature at which the GeneTAC HybStation will cycle.
- **Multiple wash** Select a solution to flow through the slide chambers for up to 10 wash steps.
- **Drain** Specify the length of time for which you want air to be drawn through the slide, to remove a solution.
- **Start loop** Create a series of steps to be repeated multiple times.
- **End loop** Complete the series of steps.

Any number of steps can be added to a protocol. When a step has been added, the **Protocol Definition** screen appears, listing the new step.

The protocol steps are described in detail on the following pages.

The Single Wash Step

This step is used to specify flow and hold times and temperatures for a wash step. The reservoir and the waste bottle to be used are also selected together with a definition of the number of times (cycles) the wash step is to be performed.

First, the modules selected for a run go to the temperature specified in the wash step. Once all modules arrive at the proper temperature, the manifolds are primed with the wash solution. The wash bottle valve and the waste valve are open at this time. For twelve slides, the two transfer valves (one for each manifold) are also open to allow flow through the manifolds without going through any slides.

Next, the wash solution flows over each slide in turn for the specified time. The maximum flow time allowed is 1 minute. For each wash, the valve for the input solution and the selected waste bottle are open for all of the slides. In addition, the particular slide's inlet and outlet valves are open for the length of time that is specified.

Then, the countdown for the hold time specified is started. All of the wash valves are closed at this stage.

The priority selected determines how the system continues. The priorities are:

- **Normal** The program may interrupt the wash cycle on one module to start and perform wash steps on other modules, moving back and forth between modules.
- **Must not be interrupted** (Critical). The program goes through the complete wash step on one module before proceeding to the next one.
- **Urgent** This step is similar to the Normal priority in that the program interrupts the wash step on one module to perform wash steps on other modules. However, when Urgent priority is selected the program next starts the wash steps for the module with this priority assigned.

The Probe Step

This step defines where in a protocol the labeled target is to be inserted into each slide of one, several, or all selected modules. The temperature at which the labeled sample is to be inserted can also be set. This temperature can be between 0 and 99°C.

When the modules have reached the specified labeled sample temperature, the manifolds are purged of their contents (that is, left-over buffer) by switching on the two purge valves, the waste valve for waste bottle one, and the air intake valve. The air intake and the waste valves are left open to allow the proper flow across the slides.

When the HybStation is within 0.25° C of the target temperature, the **Probe** screen appears, showing the appropriate slide positions.

The order in which labeled sample is added to modules is not necessarily sequential. It is dependent upon the order in which the modules reach the required temperature. It is important to be aware of which modules are ready to accept labeled sample.

When **Probe** is pressed below the first slide position; a check mark appears next to the probe button to show it is selected and the inlet valve for the slide is opened. Labeled sample is then added through the port by means of a standard 200µl pipette with disposal tips (yellow).

When this has been done, insert the labeled sample plug and press **Finished** next to the **Probe** button. The inlet valve for the slide is then closed. This process is then repeated for the second slide position, and others. As prompted, add the labeled sample to each slide in turn by module.

When labeled sample had been added to all slides, **Finished** is pressed at the bottom of the screen to move on to the next protocol step.

The Temperature Step with Agitation

This option is used to define a temperature at which the unit will cycle and to set a time for the unit to remain at that temperature. It is recommended to use this option immediately after the probe insert step to allow proper hybridization of the sample. The time can be set from 1 second up to 72 hours.

The modules are brought to temperature and time countdown starts when a module reaches the specified temperature.

In this cycle, the agitation option can also be actioned. When selected, this option automatically opens and closes the agitation (or pulse) valves, allowing the labeled sample to circulate over the entire slide surface for optimal hybridization, thus reducing the possibility of local depletion.

The agitation works on a one-second open, three-seconds closed cycle for the duration of the step. Every 5 hours during this process, the slide's inlet valve and air valve are opened for 1 second to release any pressure built up in the slide module.

The Multiple Wash Step

A multiple wash step consists of several wash steps that are combined as one single step. Up to 10 wash steps can be defined under a multiple wash, and a multiple wash step can be added several times in a protocol. Only one definition of a multiple wash for a protocol can be made.

When a multiple wash step is defined, a screen similar to the Protocol Definition screen appears. This provides access to screens enabling the following to be set:

- Reservoir bottle to be used
- Waste bottle to be used
- Specific wash time and temperature
- Specific hold time at that temperature
- Number of times the wash will be performed.

Unlike the single wash step, the manifolds are not emptied when the first wash is finished before the new buffer flows into them. This results in some mixing of fluids.

No choice of scheduling is available with this step.

The Drain Step

This option is used to select the amount of time to vent and drain the slides.

The slides are drained one at a time by having their inlet and outlet valves opened. When the air inlet valve is opened, it allows airflow over each slide to the waste bottle.

Start Loop

This option is used to create and name a loop. A loop comprises a series of steps that are defined and run a specified number of times.

Using loops relieves the creation of a long protocol, as it is only necessary to list repetitious steps only once. Once a loop is named, it can be added to a protocol in the same way as other steps.

The Start Loop command sets a flag in the protocol list that indicates the beginning of the loop.

End Loop

This option is used to end a loop and specify the number of times to run the loop. When End Loop is pressed having added the desired number of steps, you are prompted to enter the name for the loop. You must enter the same name you specified when you started the loop.

The following procedures define how to implement each of the protocol steps.

Adding a Single Wash Step

A wash step has two parts:

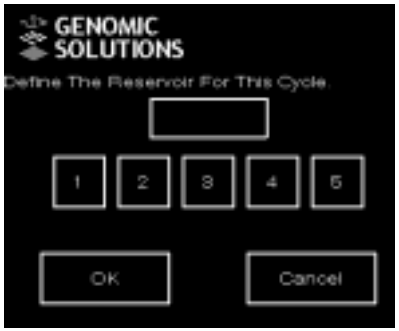
- **Flow** Fluid from a reservoir flows over the slide for a set length of time.
- **Hold** The valve closes and the chamber heats to a set temperature for a defined length of time.

When adding a wash step, select the reservoir and waste bottle, set flow time and temperature, set hold time and temperature, determine how many times the wash cycle will run, and specify the priority of the wash step.

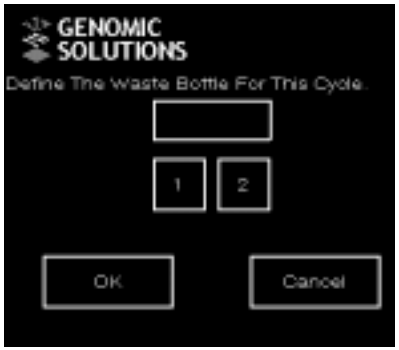
It must be remembered that the HybStation can wash only one slide at a time. The total wash time is therefore the number of minutes for one wash multiplied by the number of slides.

Note: *To return to the Protocol Definition screen without adding a single wash step, press* ***Cancel*** *on any of the screens described in the following section.*

1. On the Protocol Steps screen, press **Single Wash**. The **Define the Reservoir for this cycle** screen then opens.



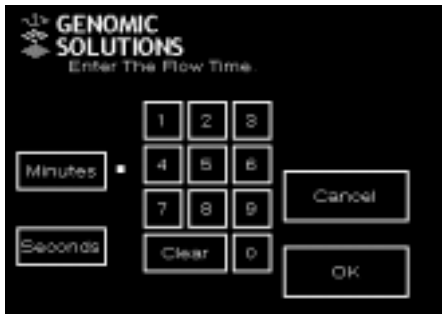
2. Press the number of the reservoir bottle to be used (1-5).
   Note: *To change the entry, press a different number. The system overrides the current entry with the new one.*
3. Press **OK**. The **Define the Waste Bottle for this cycle** screen opens. Select the waste bottle that the wash solution is to be sent to.



4. Press the number of the waste bottle to be used (1 or 2).

   Note: *To change the entry, press a different number. The system overrides the current entry with the new one.*

5. Press **OK** . A screen appears, asking for the amount of time the selected solution is to flow over the slide.



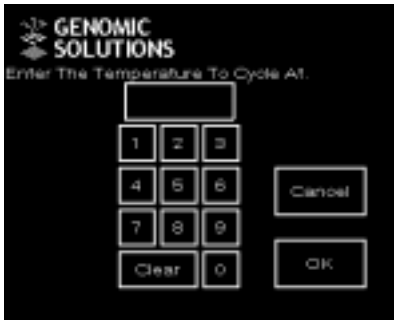
6. Enter the flow time in minutes and/or seconds.

   Note: *To select minutes,* press ***Minutes*** *then press the desired number(s). A small marker will appear next to the* ***Minutes*** *button to indicate it is selected. Similarly, to select seconds press* **Seconds**, *then press the desired number(s).*

   To change an entry, press **Clear** *and enter a different number.*

   It should also be noted that the details in this note apply to a number of the following steps in this section of the manual.

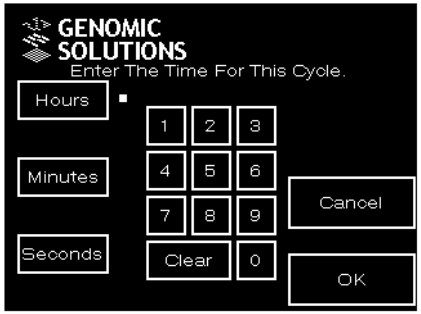
7. Press **OK**. A screen will appear prompting for the temperature at which the wash is to cycle.



8. Select the number for the desired temperature.

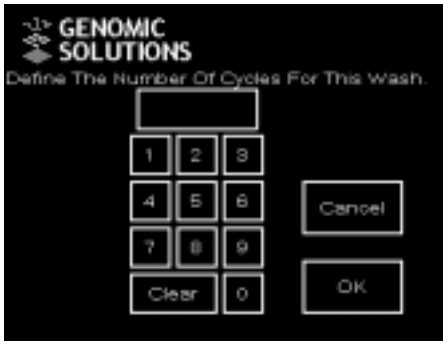
   To change the entry, press Clear *and enter a different number.*

9. Press **OK**. A screen appears prompting for the amount of time the slide is to remain at that temperature.



10. Enter the desired length of time in minutes and/or seconds.

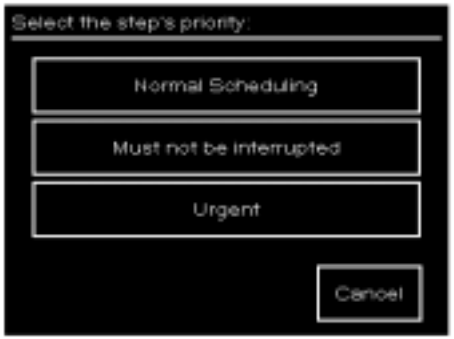
11. Press **OK.** A screen appears, prompting for the number of times this wash is to cycle.



12. Press the number of wash cycles.

Note: *The maximum number of cycles is 10. To change your entry, press* **Clear** *and enter a different number.*

13. Press **OK**. The **Select the Step Priority** screen appears.



14. Select the step priority. The preferred selection is **Must not be interrupted** .

The priorities that can be set are as follows:

- **Normal.** The program may interrupt the wash cycle on one module to start and perform wash steps on other modules, moving back and forth between modules. "Normal" can be used for a single-protocol run, but if used for a multi-protocol run the wash timing may be affected.
- **Must not be interrupted** (critical). The program goes through the complete wash step on one module before proceeding to the next one. This is the preferred choice, as it guarantees that all wash steps are performed for the exact length of time specified in the protocol.
- **Urgent.** The program runs an urgent step as soon as possible and does not interrupt the step once it has started. A step with Urgent scheduling interrupts any other steps that might currently be running but cannot be interrupted itself.

When this has been set, the **Protocol Definition** screen will appears with the new wash step displayed.

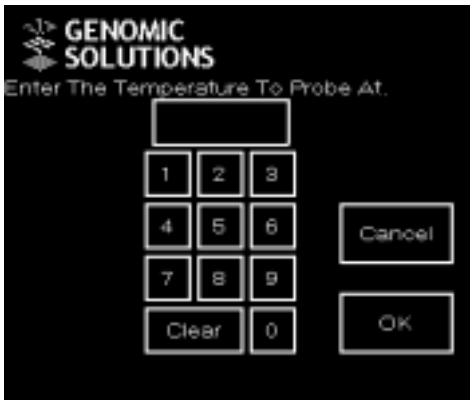
Adding a Probe (Labeled Sample) Step

This step is used to specify where in the protocol and at what temperature the labeled sample is added.

Note: *To return to the* ***Protocol Definition*** *screen without adding a Probe step, press* ***Cancel*** *on the screen.*

To add a probe step:

1. On the Steps screen, press **Probe** .
   The **Enter the Temperature To Probe At** screen then appears.



2. Enter the desired temperature.
3. Press **OK**.

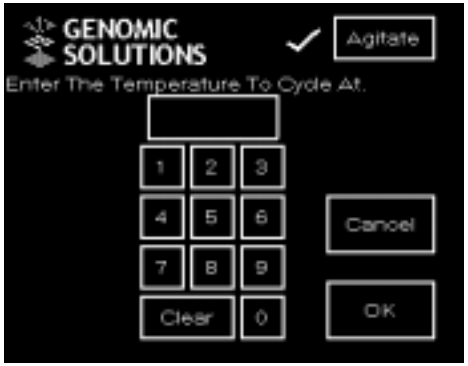
Adding a Temperature Step

This step is used to enter the temperature for hybridization and the length of time the system holds that temperature. The time can be set from 1 second up to 72 hours.

The temperature step also has an Agitate option. If selected, the system agitates the fluid on the slides during a temperature step.

To add a temperature step (hybridization step):

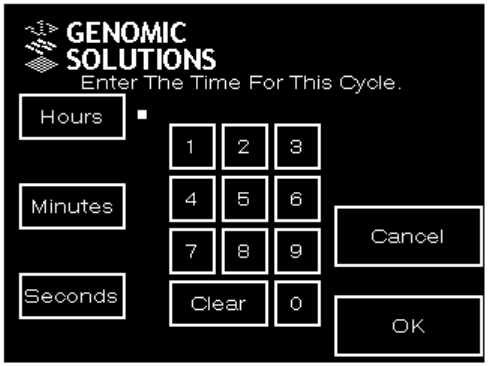
1. On the Steps screen, press **Temperature**. The *Enter the Temperature* to Cycle At screen appears.



2. Enter the desired temperature.
3. To add agitation, press the *Agitate* button at the top right of the screen. A marker indicates that agitation is selected.

   The system now automatically opens and closes valves during this step, allowing the labeled sample to circulate over the entire slide surface for optimal hybridization and to reduce the possibility of local depletion.

4. Press **OK** . A screen appears, prompting for the length of time for this step.



5. Enter the desired amount of time in hours, minutes, and/or seconds.
6. Press **OK**.

Adding a Multiple Wash Step

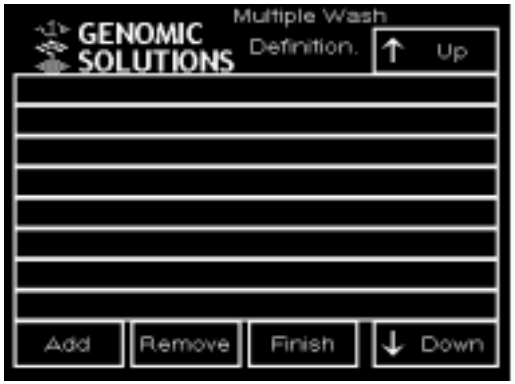
A multiple wash step is similar to a single wash step but can contain up to 10 washes within the one step.

Only one definition of a multiple wash can be made for a protocol. If a multiple wash is defined and this option is selected again, a message appears asking if the multiple wash is to be re-defined or if the current multiple wash is acceptable. If it is selected to re-define the multiple wash, either add steps can be added to your multiple wash or steps can be deleted from it.

A multiple wash consists of multiple steps, which are defined on multiple screens. To continue from one screen to the next, press the **OK** button at the bottom of a screen. If this button is pressed before making a required entry, a warning message appears prompting an entry before continuing.

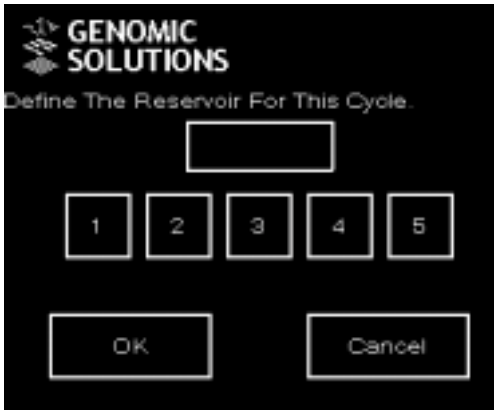
To add a multiple wash:

1. On the Steps screen, press **Multiple Wash** . The **Multiple Wash Definition** screen then appears.



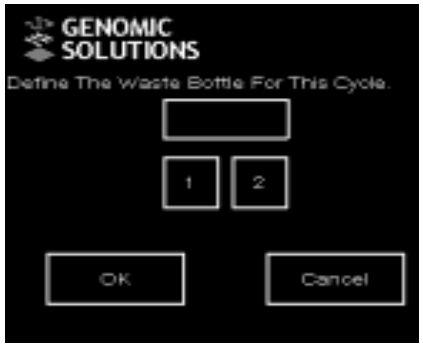
Note: *To return to the* Multiple Wash Definition *screen from any of the following screens without adding a wash step*, ***Cancel*** *can be pressed. To return to the Protocol Definition screen without adding a multiple wash step, press* ***Finish****.*

2. Press **Add**. The Reservoir screen appears, prompting you to select a reservoir bottle.

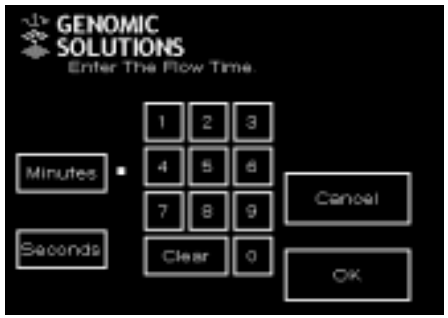


3. Press the number of the reservoir bottle to be used (1-5). The number appears in the box above the available choices. To change the entry, press a different number. The system overrides the current entry with the new one.

4. Press **OK**. The Waste Bottle screen appears, prompting for selection of a waste bottle for this cycle.



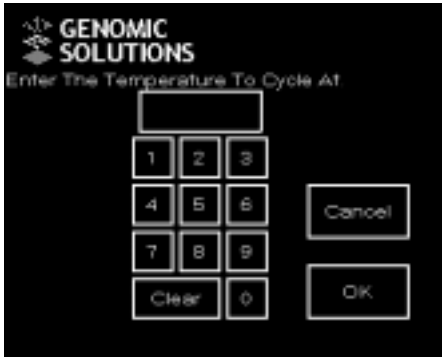
5. Press the number of the waste bottle to be used (1 or 2). The number appears in the box above the available choices. To change the entry press a different number. The system overrides the current entry with the new one.
6. Press **OK**. A screen appears, prompting for definition of the flow time for the wash.



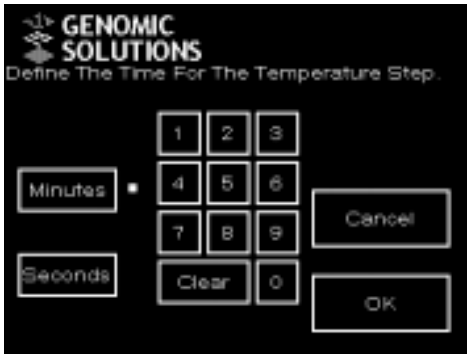
7. Enter the length of time the fluid should flow in minutes and/or seconds.

   Note: *To select minutes,* press ***Minutes*** *then press the desired number(s). A small marker will appear next to the* ***Minutes*** *button to indicate it is selected. Similarly, to select seconds press* ***Seconds*** , *then press the desired number(s).*

   To change an entry, press **Clear** *and enter a different number.*
8. Press **OK**. A screen appears prompting for a temperature step at which the wash is to cycle.



9. Press the number(s) for the desired temperature. To change the entry, press **Clear** and enter a different number.
10. Press **OK**. A screen appears, prompting for the length of time the temperature is to be maintained.

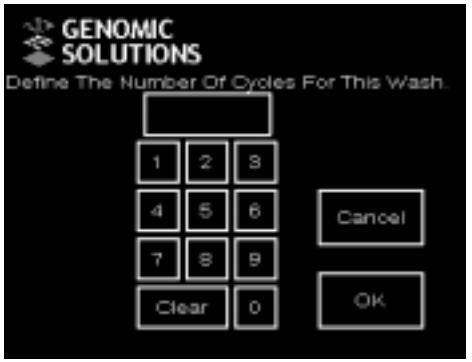


11. Enter the desired length of time in minutes and/or seconds.

    Note: *To select minutes,* press ***Minutes*** *then press the desired number(s). A small marker will appear next to the* ***Minutes*** *button to indicate it is selected. Similarly, to select seconds press* **Seconds** *, then press the desired number(s).*

    To change an entry, press **Clear** *and enter a different number.*

12. Press **OK**. A screen appears prompting for the number of times that the wash is to cycle.



13. Select the desired number of cycles. The maximum number of cycles is 10.

    To change the entry, press ***Clear*** *and enter a different number*.

14. Press **OK**. The *Wash Definition* screen appears with the wash step displayed.

15. Continue adding wash steps as desired, up to 10 steps, by repeating the previous procedures.

16. When all steps have been added to the multiple wash, press **Finish**. The Protocol Definition screen appears, listing the Multiple Wash as one step.

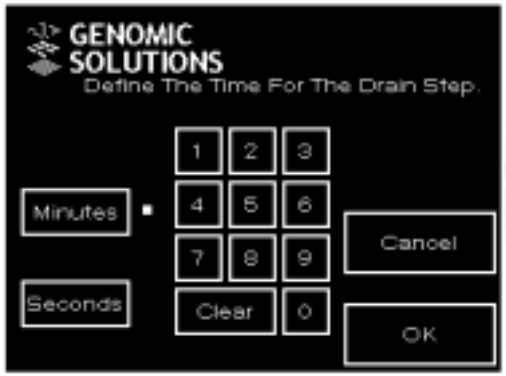
Adding a Drain Step

The drain step is used to select a specified length of time for which each slide is drained.

Note: A drain step is only used for the removal of prehybridization solution.

To add a drain step:

1. On the Steps screen, press **Drain**. The Define Drain Time screen appears.



2. Enter the amount of time that the system is to drain, in minutes and/or seconds. *The maximum time is 20 minutes.*

   Note: *To select minutes,* press ***Minutes*** *then press the desired number(s). A small marker will appear next to the* ***Minutes*** *button to indicate it is selected. Similarly, to select seconds press* **Seconds** *, then press the desired number(s).*

   To change an entry, press **Clear** *and enter a different number.*

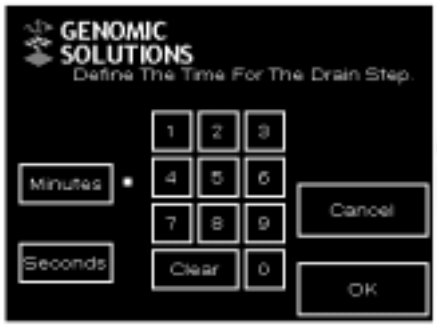
3. Press **OK**.

Adding a Loop

A loop is a series of steps that are defined and saved to use in a protocol where a number of steps is repeated several times. Rather than adding all these steps repeatedly, they can be saved as a loop that forms part of the final protocol.

To create a loop:

1. On the *Steps* screen, press **Start Loop** . The Keypad screen appears, prompting for a name to be entered for the loop.



2. Enter a name of up to eight alphanumeric characters using the alphanumeric buttons.

   Note: *To delete a character, press* Bkspc *.*
   To cancel the operation and return to the Protocol *screen, press* ***Cancel****. To accept the entry and continue, press* ***OK*** *.*

   The Protocol Definition screen will then appear showing a new step called Start loop xxx, where xxx is the name defined for the loop.

3. Press **Add**. The *Steps* screen then appears.

4. Add steps until all desired steps are listed on the Protocol Definition screen.

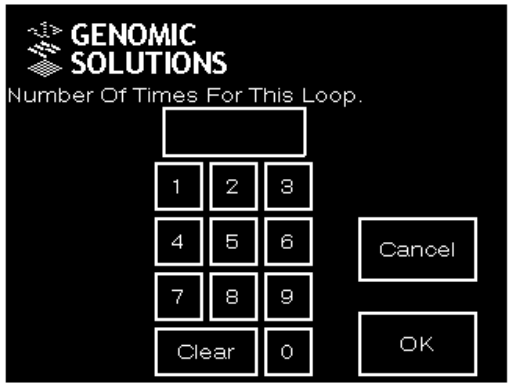
5. On the Steps screen, press **End Loop** . A Keypad screen then appears, prompting for the name of the loop.

6. Enter the same name for the loop previously entered in step 2. *The system then checks the name to make sure it corresponds with a start loop*.

   Note: *If the correct loop name is not entered, a message appears informing that the name is not valid and to please try again.*

7. To return to the Keypad screen, press **OK**.

   When a valid loop name has been entered a screen appears prompting for the number of times the loop is to be executed.



8. Enter a number from 1 to 10. The Protocol Definition screen then appears with the loop step listed.

Section 4

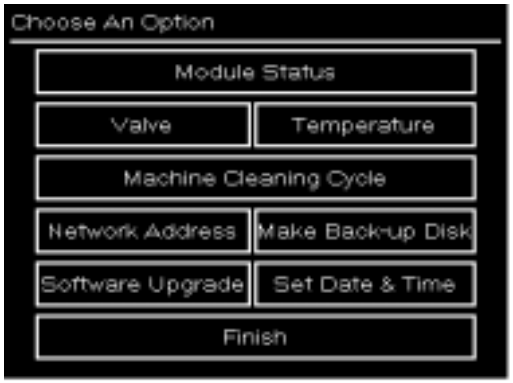
Maintaining the System

Accessing the Maintenance Screen

All maintenance options are accessed through the Maintenance (Choose an Option) screen.

To open the Maintenance screen:

On the Main screen, press **Maintenance**. The Maintenance screen then opens.



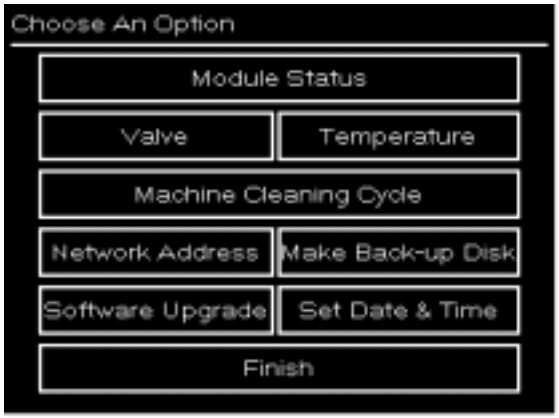
To return to the Main screen, press **Finish** .

Checking a Module Temperature

Module temperatures can be checked using the Module Status screen. The screen lists the current temperatures of the six modules.

To view current module temperatures:

On the Maintenance screen, press Module Status . The Module Status screen opens.



To return to the *Maintenance* screen, press *Close* .

Testing the System Valves

A number of valves are incorporated into the HybStation systems.

Each slide has three dedicated valves:

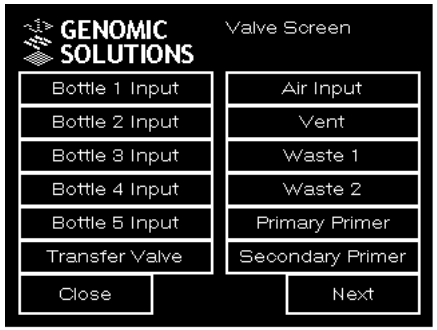
- Inlet Valve
- Agitation Valve
- Outlet Valve

Additionally, there are bottle and air inlet valves, waste valves, vent and transfer valves, and valves for the manifolds, called primary and secondary primers.

The **Valve Screen** is used to manually open and close valves and run liquid through them.

To test selected valves:

1. Place tubes 1 through 5 into a single container of ultrapure water.
2. On the Maintenance screen, press **Valve**. The first **Valve Screen** appears.

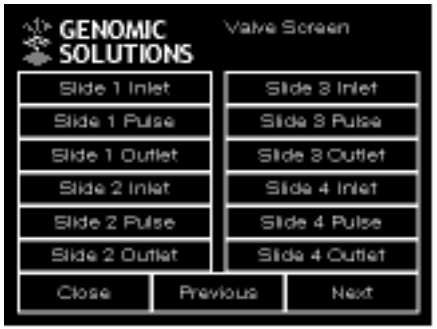


3. Select the valve/s to be tested.

Note: *It must be ensured that an input valve is not selected for an empty or non-existent bottle (reservoir). A check mark appears next to each selected valve.*

4. Observe the flow of the liquid.

To select valves for individual slides, press **Next** at the bottom of the screen. The second **Valve Screen** will then appear, listing all three valves for slides 1 through 4.



Select the valve/s to be tested. *A check mark appears next to each selected valve.*

To return to the first Valve screen, press **Previous**. To select valves for further slides, press **Next**.

To close a Valve screen and return to the Maintenance screen, press **Close** .

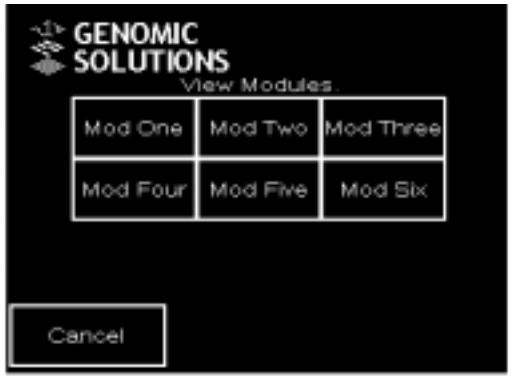
To stop testing the valves and return to the Main screen, press **Finish** on the Maintenance screen.

Note: *Always ensure that all valves are turned OFF before closing the maintenance screen.*

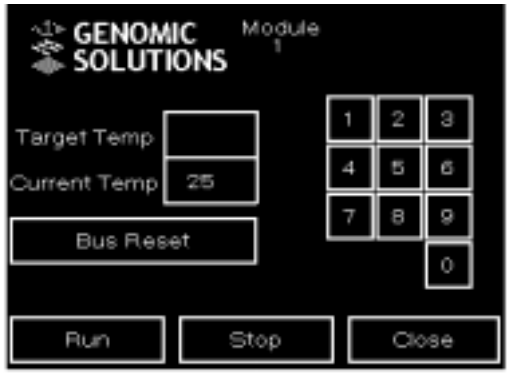
Testing the Heating Element of a Module

The heating element of a module is tested as follows

1. On the Maintenance screen, press **Temperature**. The **View Modules** screen opens.



2. Select a module on which to test the heating element. A Temperature screen then opens for the module.



3. Enter a target temperature that is higher or lower than the current temperature using the keypad on the right-hand side of the screen.
4. Press **Run**.
5. Observe the temperature increase or decrease.
6. Press **Stop** to enter a new target temperature and use the keypad to enter the new temperature.
7. Press **Close** to stop the test and return to the *Maintenance* screen.

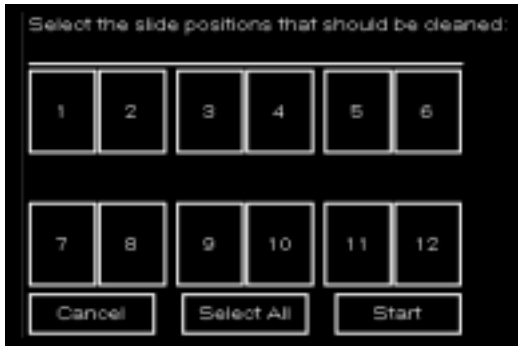
Bus Reset can be pressed to reset data transmission with the module should any communications problem occur.

Cleaning the Valves and Lines

A machine cleaning cycle should be performed on modules that were used after each run. The water in this cycle removes solutions to prevent precipitation in the valves and lines. The cleaning program allows the selection of slide positions for cleaning.

To clean the valves and lines:

1. On the Maintenance screen, press **Machine Cleaning Cycle** . The Slide Positions screen will then open.



2. To select specific slides, press the slides on the screen. To select all slides, press **Select All** .

3. To start the cleaning cycle, press **Start** . A message appears, prompting preparation of the HybStation by:
   - Placing tubes 1 through 5 into a single container of ultrapure water
   - Fitting slides at all positions selected for cleaning

4. Press **Continue**. The cleaning cycle will then start.

   Note: *To maintain a clear fluidic system it is good to do a Machine Clean cycle using warm water at approx. 85°C every month.*

Changing the Network Address

If multiple HybStations are being run in a network, each instrument must have a unique network address.

The **Network Address** screen is used to change the network address of the GeneTAC HybStation.

To change the network address:

1. On the Maintenance screen, press **Network Address** . The Network Address screen appears:

2. Use the up arrow and down arrow buttons to change the network address displayed to the left. Use "1" for the instrument closest to the controlling computer.
3. Press **OK** to return to the Maintenance screen.

Making a Back-up Disk

Make a backup copy of the protocols on a disk using the Make Back-up Disk screen.

To make a backup disk:

1. Insert an IBM-formatted floppy disk into the disk drive.
2. On the Maintenance screen, press **Make Back-up Disk**

   A message screen opens:

3. Press **Start**

Upgrading the Hybstation Software

Software for the HybStation must be upgraded when a new version is released.

To upgrade the software:

1. Insert the floppy disk that contains the software upgrade.
2. On the Maintenance screen, select **Software Upgrade** and follow the instructions on the screen.

Note: *Be certain to insert the disk before pressing* ***OK*** *on the second screen. If the disk is not inserted when OK is pressed, turn off the HybStation and repeat the process*.


Use the arrows to set the time and date:
09:08 - 16 May 2000
Currently editing: Hours
Cancel
OK

Setting the Date and Time

The date and time for the HybStation at the Set Date and Time screen.

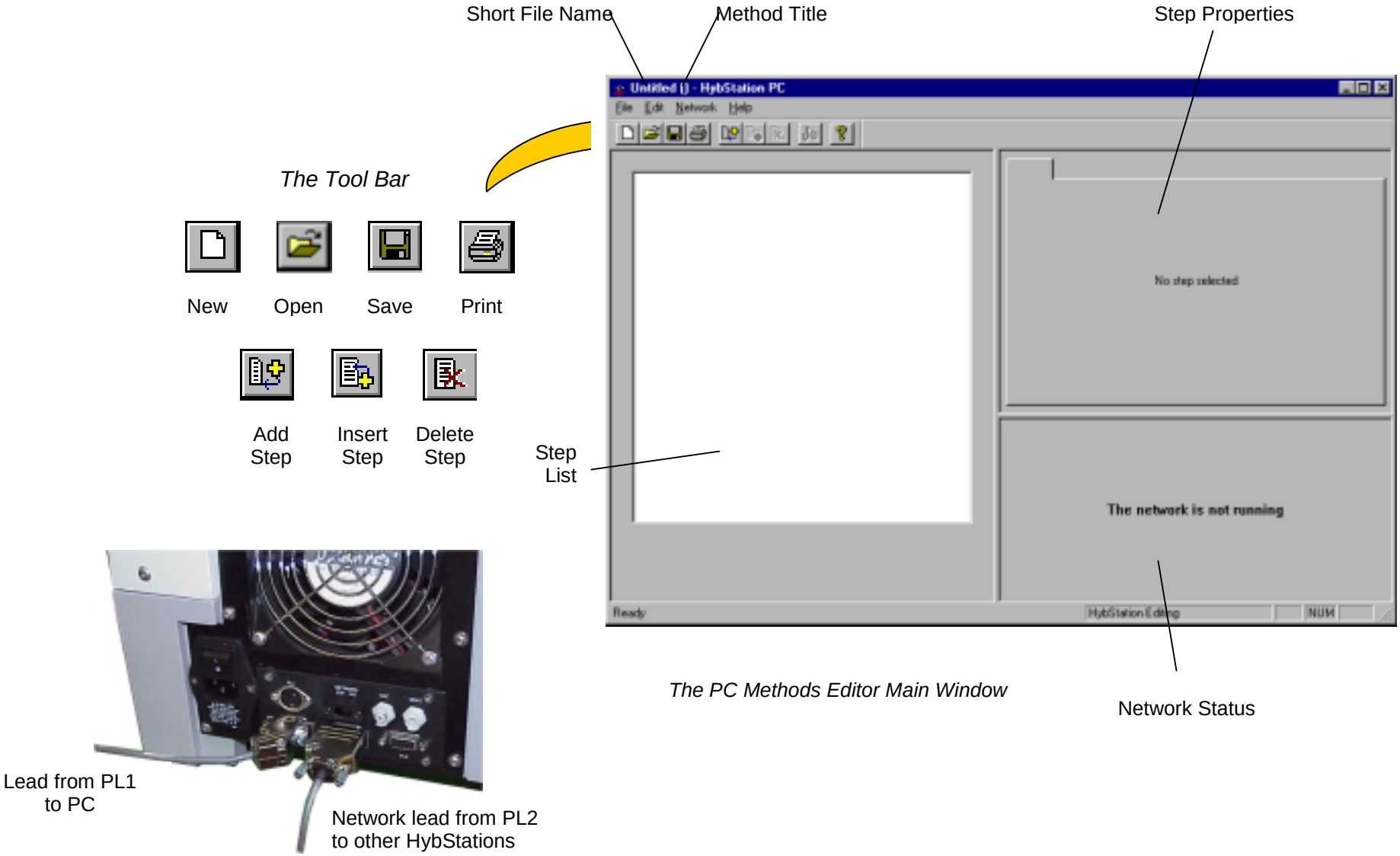
To set the date and time:

1. On the Maintenance screen, select **Set Date & Time**. The Date and Time screen opens.
2. Press the left and right arrows to edit the year, month, day, hours, or minutes when the selection is in the currently editing field.
3. Press the up and down arrows until the correct numbers appear near the top of the screen.
4. To accept the new settings and return to the Maintenance screen, press **OK** .

   To return to the Maintenance screen without accepting the new settings, press **Cancel** .

Section 5

Using the PC Methods Editor Software



The PC Methods Editor Main Window

View of HybStation Rear Panel

Introduction to the PC Methods Editor Software

The PC Methods Editor software runs on a PC and can be networked to the HybStation. Using the software, protocols can be created, edited, and printed.

To connect the HybStation to a PC, a communications lead (Part No. H2001016) is used between port PL1 on the HybStation and the PC, as shown above.

To network more than one HybStation together, a communications lead (Part No. H2001013) is used between the PL2 ports on the connected HybStations.

Installing the PC Methods Editor Software

The software for the PC Editor is available from Genomic Solutions and is installed as follows:

1. Install the software by copying the self-installing file to the PC and executing it.
2. At the PC navigate to the folder where the program is installed.

   C:\ProgramFiles\GenomicSolutions\HybStationPC.
3. Launch the program by double-click the icon.

   The Main window will then appear.

Working in the PC Editor

The software is easy to use and is controlled from the Main window.

The main window comprises:

- **The Menu Bar** providing access to functions for working with protocols via menus.
- **The Toolbar** provides access to functions for working with protocols via buttons.

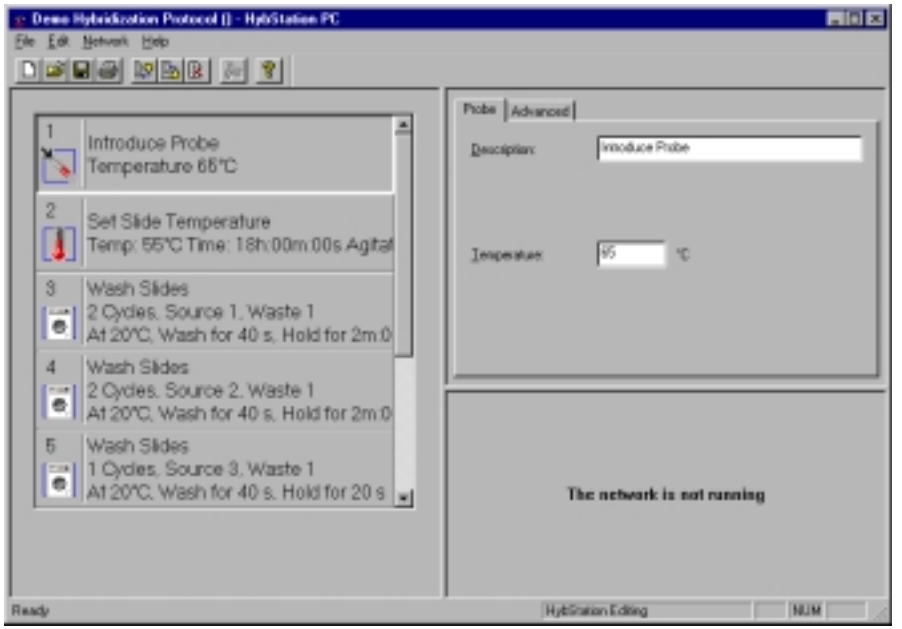
  As shown on the above figure, the first four toolbar buttons are standard Windows program buttons and the next three buttons are used when creating or editing a protocol:
- The **Step List** area shows a list of all the steps in the current protocol.

  Note: *To select a step, left-click on it.*
- The **Step Properties** area shows the properties of a selected step.

The Network Status area, used when networking, shows all the Genomic Solutions devices connected to the PC including the instrument type and its status.

Opening a Protocol

A protocol is opened through the **File** menu or by using the toolbar button.



1. To open a protocol, either:
   - From the **File** menu, choose **Open** .
   - On the tool bar, click the Open button.
2. The standard Browse dialog box will then open.
3. Browse to the location where the protocol is stored. This can be the PC hard drive or a floppy disk.
4. Select a protocol and click **Open** .

   The Main window will then open showing the protocol steps.

   At the Main window the step list (on the left) shows all steps in the protocol, and the step properties are shown in the view area on the right.

To view the properties of any step, click on the step.

To view additional step details of step properties, click the **Advanced** tab.

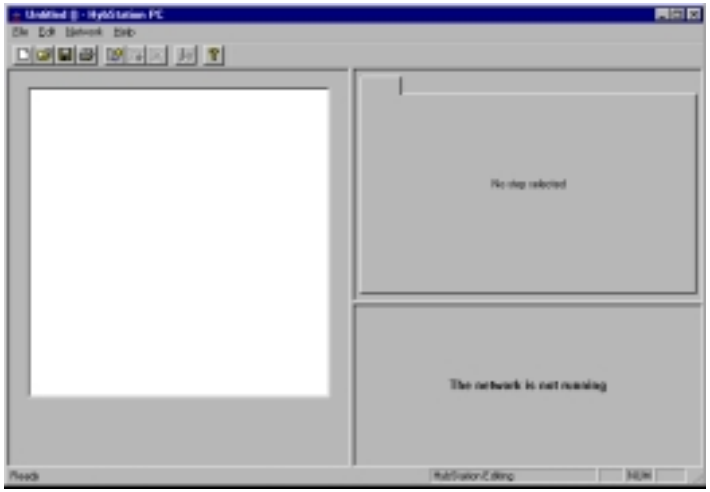
Creating a New Protocol

When the PC Methods Editor software first opens, the main screen has a blank step list. Steps are then added to create a new protocol.

To create a new protocol, proceed as follows:

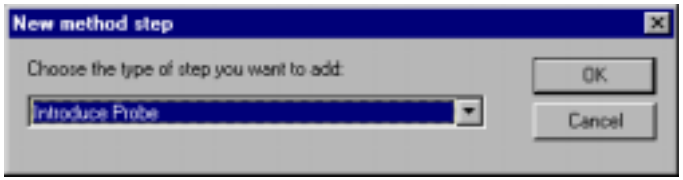
1. Start a new file. To do this either select **New** from the File menu or on the toolbar click the New button.

The Main software window will then appear, having a blank step list.

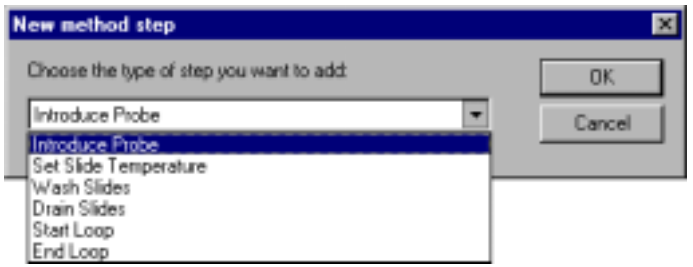


2. To add a step to the new protocol, select **Add Step** from the Edit menu or click on the **Add Step** button on the toolbar.

The **New Method Step** dialog box will appear.



3. From the Steps drop-down list, select the step to be added. *All available steps will appear in the list.*



4. Click **OK**.
5. Repeat the above procedures to add additional steps to the protocol.
6. From the File menu, choose **Save**. The **Save As** dialog box will then appear, allowing the new protocol to be saved.

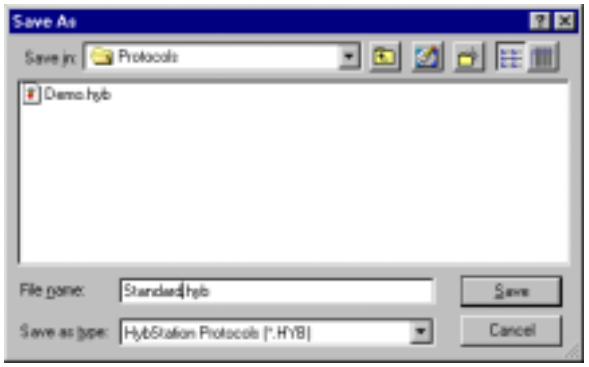
   Note: *For additional information refer to the next section, Saving a Protocol.*

Saving a Protocol

Changes to a protocol can be saved under the current protocol name or using a new name and create a protocol. When you create a new protocol, you can save it to any directory under any name.

To save a protocol under a new name:

1. From the File menu, choose **Save As.** The **Save As** dialog box appears.



2. Browse to the directory where the protocol is to be saved.
3. In the **File name** field, enter a name for the protocol.

Note: *Because the HybStation does not permit the use of long file names, use a name with 8 characters or fewer.*

4. Click **Save** or press the Enter key. The software will then save the protocol with a .HYB extension.

To save changes to a protocol, do one of the following:

- From the File menu, choose **Save**.
- On the toolbar, click the Save button.

If the HybStation is not networked to a PC, a protocol can be saved onto a floppy disc and transferred to the HybStation.

Editing a Protocol

A protocol can by edited by adding, inserting, or deleting steps.

Note: *It must be noted that a protocol created using the methods editor software on the HybStation cannot be edited using this program.*

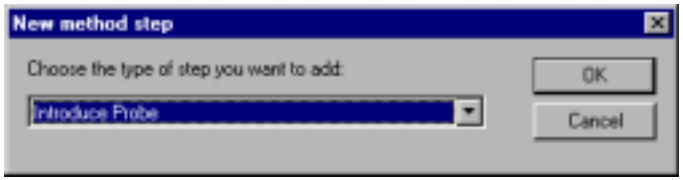
Adding Steps to a Protocol

When a step is added to a protocol, PC Methods Editor software places the steps at the end of the protocol.

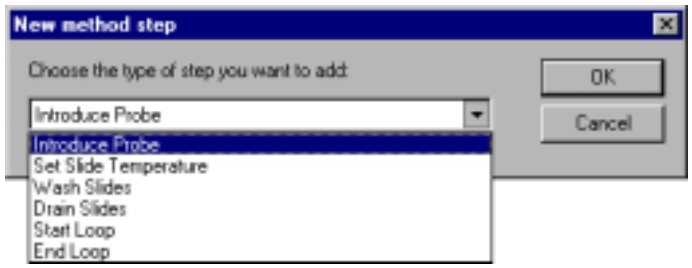
To add a step to an existing protocol:

1. Open a protocol. The Main window will then appear, showing the steps for the protocol.
2. Do one of the following:
   - From the Edit menu, choose **Add Step**.
   - On the toolbar, click the Add Step button.

The **New Method Step** dialog box will then appear.



3. From the Steps drop-down list, select the step to be added. All available steps are shown in the list.



4. Click **OK**.
5. Repeat Steps 2 through 4 to add additional steps to the protocol.
6. Save the changes to the protocol:
   - To save the changes using the same protocol name, choose **Save** from the File menu.
   - To save the changes using a different protocol name, choose **Save As** from the File menu.

   Note: *Always use a file name with 8 characters or less, or make the first 8 characters recognizable.*

Inserting Steps into a Protocol

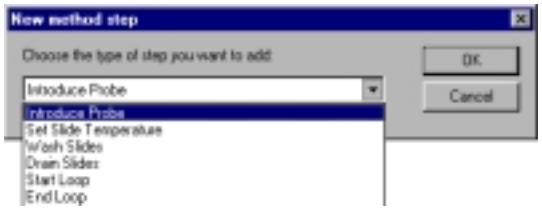
When a step is inserted into a protocol, it will be positioned before a selected step in the protocol.

To insert a step into a protocol:

1. Open a protocol. The Main window will then appear, showing the steps for the protocol.
2. In the step list, click the step before the location of the new step.
3. Do one of the following:
   - From the Edit menu, choose **Insert Step**.
   - On the toolbar, click the Insert Step button.

   The **New Method Step** dialog box will then open.

4. From the *Steps* drop-down list, select the step to be inserted. *All available steps will be shown in the list.*



5. Click **OK**.
6. Repeat Steps 2 through 5 to insert additional steps into the protocol.
7. Save the changes to the protocol:
   - To save the changes using the same protocol name, choose **Save** from the File menu.
   - To save the changes using a different protocol name, choose **Save As** from the File menu.

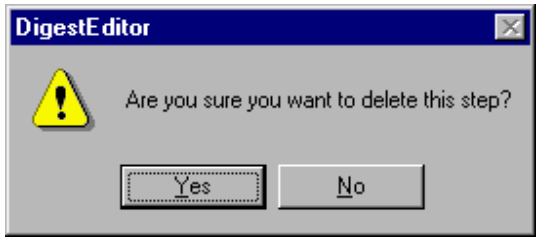
   Note: *Always use a file name with 8 characters or less, or make the first 8 characters recognizable.*

Deleting Steps from a Protocol

To delete a step from a protocol:

1. Open the protocol.
2. The Main window will then appear, showing the steps of the protocol.
3. In the step list, click on the step to be deleted.
4. Do one of the following:
   - From the *Edit* menu, choose *Delete Step* .
   - On the toolbar, click the *Delete Step* button.

   A Confirmation dialog box will then appear :

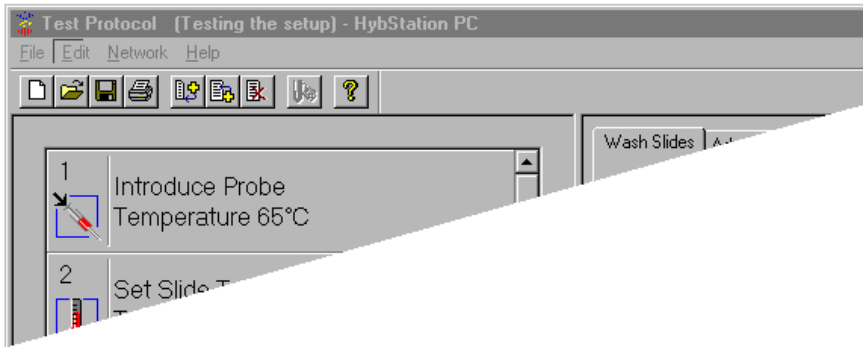


5. Click *Yes*.
6. Save the protocol changes:
   - To save the changes using the same protocol name, choose **Save** from the File menu.
   - To save the changes using a different protocol name, choose **Save As** from the File menu.

Note: *Always use a file name with 8 characters or less, or make the first 8 characters recognizable.*

File Name

Method Title – *description of protocol*



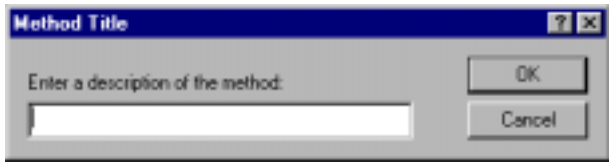
Setting a Method Title

The method title is the name that appears in parentheses at the top of the protocol window, next to the protocol name.

The method title is used to add a description of the protocol to the file name.

To set a method title for a protocol:

1. Open the protocol.
2. From the **File** menu, choose **Set Method Title**. The **Method Title** dialog box appears. Enter a description of the protocol.



3. Type a description of the protocol.
4. Click **OK** or press the **Enter** key.

To change the method title for a protocol:

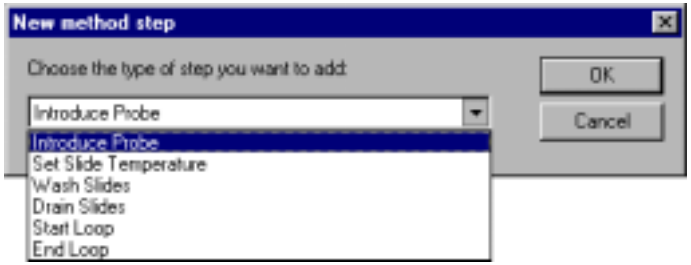
1. Open the protocol.
2. From the File menu, choose **Set Method Title**. The **Method Title** dialog box appears.
3. In the text field, highlight the current entry, then type a new description for the protocol.
4. Click **OK** or press the **Enter** key.

The Protocol Steps

Introduction

Steps can be added to a protocol in the PC Editor by selecting them from the **New Method Step** drop-down list, then setting durations, temperatures, and priorities.

The *Steps* drop-down list offers the following choices:



- **Introduce Probe** Specify the temperature at which the labeled sample is added.
- **Set Slide Temperature** Specify the temperature of the slides.
- **Wash Slides** Select the wash solution(s), temperature, and flow time.
- **Drain Slides** Specify the amount of time air should be drawn over the slide to remove a solution.
- **Start Loop** Create a series of steps that is repeated multiple times.
- **End Loop** Select the number of repetitions of the series of steps.

Additional details about the functions of these steps are provided later in this section.

To display the Steps drop-down list, carry out one of the following:

- From the *Edit* menu, choose *Insert Step* .
- From the *Edit* menu, choose *Add Step* .
- On the toolbar, click the *Insert Step* button.
- On the toolbar, click the *Add Step* button.

Scheduling

The timing of protocol steps is controlled by scheduling. This is set when each step is defined.

Each module on the HybStation runs independently. If all the steps are set to Normal scheduling, the GeneTAC HybStation performs the steps concurrently for each module. For example, if Modules 1 through 4 are selected for a protocol that has three steps, the system performs Step 1 on all 4 modules, then Step 2, etc. But some resources required by the steps use parts of the station exclusively. For example, the high power input required by the Peltier unit when heating the slides. As a result of this, only one module can use the resource at a time, while the other modules have to wait.

In some cases, the time spent waiting for HybStation resources may not affect the experiment being carried out. There are cases, however, where a wait after a certain step may affect the experiment results. For example, a wash step flushes solution through the slide and holds it for a given time. When the hold time begins, the manifold is available for another step to use and might not be available to implement a second wash on the particular slide. As a result, the wash solution may be held longer than anticipated, which may affect the outcome of an experiment.

To avoid potential problems of this type, Critical or Urgent scheduling can be employed. If this is used, it must be considered how this scheduling will affect the other modules, as follows:

- The HybStation can implement steps with Normal scheduling on all modules at the same time, depending on the procedures required by the step. This is the most efficient use of the processing time.
- If Critical or Urgent scheduling is used, the current step ties up the HybStation because no other step can run at the same time. This is the least efficient use of the processing time but may be necessary for the processes involved.

Three scheduling options are available, as follows:

Normal

Critical (This step must not be interrupted)

Urgent (This step will be run as soon as possible)

When a step finishes processing on any of the modules, the HybStation looks to see which step or steps it can start next. If a step with Urgent scheduling is ready to start, it takes priority, followed by steps with Critical scheduling, and lastly by steps with Normal scheduling.

Normal Scheduling

Normal is the default scheduling setting. The program may interrupt a step on one module to start and perform steps on other modules, moving back and forth between modules. When Normal scheduling is selected for a wash step, for example, there is no guarantee that the temperature steps within the wash step will be performed for the exact length of time determined by the protocol.

Normal scheduling should therefore be applied to steps such as pre-conditioning the slide, the pre-hybridization wash, the introduction of a labeled sample, or the hybridization period.

Critical Scheduling

Before performing a step with Critical scheduling, the HybStation checks that all resources are available before starting the step so it can go through the complete step on one module before proceeding to the next one. This is the preferred choice, since it guarantees that all wash steps are performed for the exact length of time specified in the protocol.

Once a step with Critical scheduling has started, it ties up the whole station, and no other step can run at the same time.

If several steps are to run consecutively, specify those steps with Critical scheduling. In that case, the HybStation works through all these steps without interruption.

Urgent Scheduling

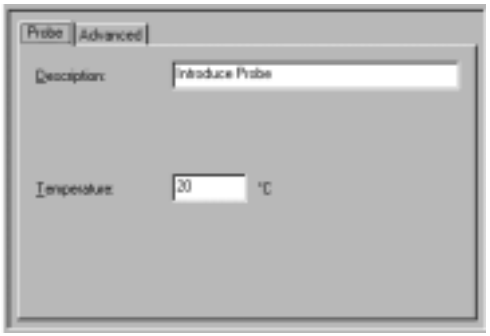
The program runs an Urgent step as soon as possible and does not interrupt the step once it has started. A step with Urgent scheduling interrupts Normal steps that are currently running. An Urgent step cannot interrupt a Critical step, but can supersede it.

The Introduce Probe (Labeled Sample) Step

This step is used to specify where in the protocol and at what temperature the labeled sample is added. The temperature can range from 1° to 99° C.

To introduce a labeled sample:

1. From the Steps drop-down list, choose **Introduce Probe**. In the Main window, a labeled sample step is added on the left, with the corresponding tabs on the right-hand side.



2. On the Probe tab:
   - Optionally, enter a brief description for this step in the protocol by highlighting the current entry and typing a new one. The description will replace the name of the step in the step list.
   - Enter the temperature at which it is required to introduce the labeled sample, by highlighting the current entry and typing a new one.

The Set Temperature Step

This step is used to set the temperature for hybridization and the length of time for which the system will cycle at that temperature.

To set the hybridization temperature:

1. From the Steps drop-down list, choose Set Slide Temperature. In the Main window, a temperature step is added on the left, with the corresponding tabs on the right.

2. On the **Temperature** tab:

- ❑ Optionally, enter a brief description for this step in your protocol by highlighting the current entry and typing a new one. The description replaces the name of the step in the step list.
- ❑ Enter the desired temperature by highlighting the current entry and typing a new one.
- ❑ Enter the length of time for the temperature step in hours (H), minutes (Min), seconds (Sec).
- ❑ Click the ***Advanced*** tab.

2. On the **Advanced** tab:

- ❑ To add agitation, make sure **Enable Agitation** is checked. The system will then automatically open and close the agitation valves during this step, allowing the labeled sample to circulate over the entire slide surface for optimal hybridization and to reduce the possibility of local depletion.
- ❑ Select the required scheduling for this step.

The Wash Slides Step

This step is used to select a solution from one of the five available reservoirs to flow through the slide chambers for a specified period of time, at a specified temperature.

To define a Wash Slides step:

1. From the Steps drop-down list, choose **Wash Slides**. In the Main window, a wash slides step is added on the left, with the corresponding tabs on the right.

2. On the **Wash Slides** tab:
   - ❑ Optionally, enter a brief description for this step in the protocol by highlighting the current entry and typing a new one. The description replaces the name of the step in the step list.
   - ❑ From the **Source** drop-down list, select one of the five reservoirs.
   - ❑ From the **Waste** drop-down list, select a waste bottle for the solution.
   - ❑ Define a temperature by highlighting the current entry and typing a new one.
   - ❑ Define a flow time and a hold time by highlighting the current entries and typing in new ones.
   - ❑ Click the ***Advanced*** tab.

3. **On the Advanced** tab:
   - ❑ Set the number of times you want the wash to cycle by highlighting the current entry and typing a new one.
   - ❑ Select the scheduling for this step.

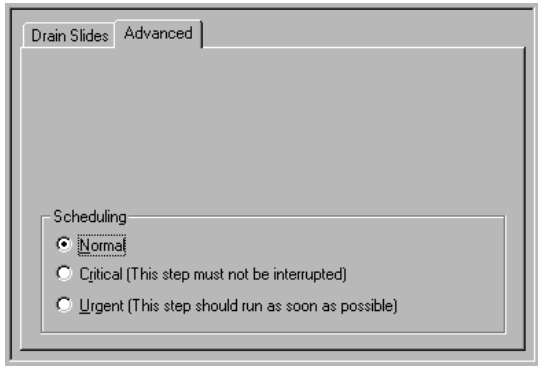
The Drain Slides Step

This step is used to specify the length of time that each slide is drained.

To define a drain step:

1. From the Steps drop-down list, choose **Drain Slides**. In the Main window, a drain slides step is added on the left, with the corresponding tabs on the right.

2. On the **Drain Slides** tab:
   - Optionally, enter a brief description for this step in the protocol by highlighting the current entry and typing a new one. The description replaces the name of the step in the step list.
   - From the **Waste** drop-down list, choose a waste bottle.
   - Specify the length of time that slides are to drain by highlighting the current entry and typing a new one.
3. Click the **Advanced** tab.



4. On the **Advanced** tab, select scheduling for this step.

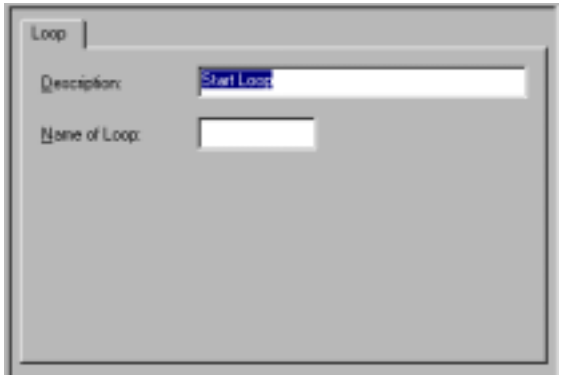
Creating a Loop

A loop can be created when a series of steps are repeated in a protocol. A loop must have a Start Loop step at the beginning and an End Loop step at the end of the series. The number of cycles that the series is repeated in defined in the End Loop step.

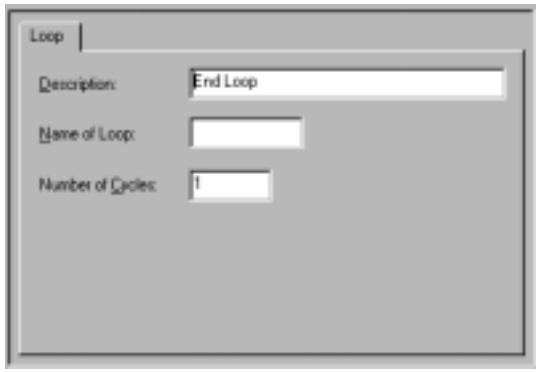
A nested loop is created when a series of steps are repeated within another loop. Each nested loop must also include a Start Loop step and an End Loop step.

To create a loop:

1. From the Steps drop-down list, choose **Start Loop**. In the Main window, a start loop step is added on the left, with the **Loop** tab on the right.



2. On the tab:
   - ❑ Enter a brief description of this step by highlighting the current entry and typing a new one. The description replaces the name of the step in the step list.
   - ❑ Enter a name for the loop.
   - ❑ Add steps to the loop.
   - ❑ From the Steps drop-down list, choose **End Loop**. In the Main window, an end loop step is added on the left, with the End Loop tab on the right.



3. On the tab:
   - ❑ Enter a brief description of the step by highlighting the current entry and typing a new one. The description replaces the name of the step in the step list.
   - ❑ Enter the loop name that was entered on the **Start Loop** tab.
   - ❑ Enter the number of cycles that the loop is required to run for.

Printing a Protocol

An open protocol can be printed out to review the protocol.

To print a protocol:

1. Ensure that the protocol to be printed is open.
2. Do one of the following:
   - From the File menu, choose **Print**.
   - On the toolbar, click the Print button.
3. On the Print dialog box, click **OK**.

   Note: *To see what a printed protocol would look like choose* **Print Preview** *.*
4. Click **OK**.

Section 6

Example of a
Typical Protocol

Example of a Typical Protocol

This section shows an example of a typical protocol.

	Step	Description
1	**Probe**	*Temperature: 65°C*
2	**Temperature**	*Temperature: 55°C* *Time: 18 hours* *Agitate: Yes*
3	**Single Wash**	*Reservoir: 1* *Waste Bottle: 1* *Flow Time: 10 Seconds* *Temperature: 55°C* *Temperature Time: 20 seconds* *Cycles: 3*
4	**Single Wash**	*Reservoir: 2* *Waste Bottle: 1* *Flow Time: 10 Seconds* *Temperature: 25°C* *Temperature Time: 20 seconds* *Cycles: 3*

5	**Single Wash**	*Reservoir: 3* *Waste Bottle: 1* *Flow Time: 10 seconds* *Temperature: 25°C* *Temperature Time: 20 seconds* *Cycles: 3*

During this typical protocol the reservoirs should contain the following GeneTAC Wash Buffer Solutions:

Reservoir 1: Medium Stringency Wash Buffer
Reservoir 2: High Stringency Wash Buffer
Reservoir 3: Post Wash Buffer