

GENEMACHINES®

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**HydroShear® DNA
Shearing Device**

User Manual

HydroShear® DNA Shearing Device

Serial Number	
Software Version	
Owned By	
Date Installed	
Service Contract Type	
Service Contract Dates	

Service Record

Service Record			

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1. About the HydroShear® DNA Shearing Device

The Hydroshear DNA Shearing Device is an automated, Point-sink Shearer (PtS), offering a simple, reproducible, and controllable method of fragmenting DNA. The Hydroshear DNA Shearing Device creates and controls hydrodynamic forces that work in conjunction with its innovative shearing assembly to consistently and repeatedly shear DNA. A wide range of DNA samples are compatible with the Hydroshear DNA Shearing Device, and the shearing parameters can be adjusted to produce specific fragment lengths of DNA.

1.1 THE COMPONENTS

Each Hydroshear DNA Shearing Device comes complete with the following:

- HydroShear DNA Shearing Device base unit
- 500 µL syringe
- Four #025 shearing assemblies
- Power cord
- RS-232 cord and connectors
- Software
- Tool kit

1.2 TECHNICAL SPECIFICATIONS

- **Fuse Current Rating:** 3 Amps
- **Fuse Voltage Rating:** 250V
- **Voltage:** 100-250 VAC
- **Frequency Range:** 47-100 Hz
- **Installation (Over voltage) Category:** II

1.3 FACILITY AND HARDWARE REQUIREMENTS

1.3.1 Electrical Requirements

- One properly grounded 110V / 15 amp or 220V / 10 amp wall outlet

1.3.2 Computer/Software Requirements

The Hydroshear DNA Shearing Device software can be installed on any PC fulfilling the following requirements:

- IBM-compatible
- Operating system: Microsoft Windows 3.11, 95, 98, or NT
- One serial port available to connect to the Hydroshear DNA Shearing Device base unit

1.3.3 Environmental Requirements

- **Pollution Degree:** 2
- **Installation Category:** II
- **Altitude:** any up to 2000 m
- **Temperature Operating (mechanism):** 59°F (15°C) to 104°F (40°C)
- **Humidity Operating (mechanism):** 20-95%RH at 104°F (40°C)

1.4 CONTACTING GENEMACHINES®

Please contact GeneMachines directly with questions about operating and maintaining the Hydroshear DNA Shearing Device or if your Hydroshear DNA Shearing Device requires servicing. To help us assist you, please provide the following information:

- The unit serial number (located on the machine's back panel)
- The name of your company or institution
- Your name and contact information
- A full description of the problem

About the HydroShear® DNA Shearing Device

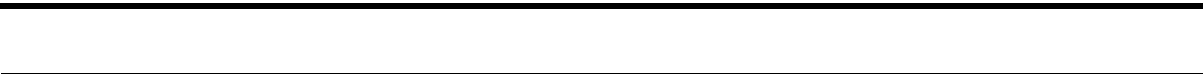
Phone: 1-877-855-GENE (4363), 1-650-508-1634

Fax: 1-650-508-1644

E-mail: support@genemachines.com (please include “Hydroshear DNA Shearing Device” in subject line)

Address:

GeneMachines
Attn. Hydroshear DNA Shearing Device Customer Support
935 Washington Street
San Carlos, CA 94070



2. HydroShear® DNA Shearing Device Design and Shearing Method

As the DNA samples are accelerated through a narrow orifice, drag forces shear the DNA molecules apart.

2.1 DESIGN OF THE HYDROSHEAR DNA SHEARING DEVICE

The innovative design of the HydroShear DNA Shearing Device appropriates hydrodynamic forces and a one-way, flow-through orifice to fragment DNA. This design is based on the hydrodynamic point-sink shearing method originally developed by Oefner et al. (1996)¹; “point-sink” refers to a theoretical model of the hydrodynamic flow through such a system.

2.1.1 Shearing Orifice

In order to accomplish accurate, reproducible shearing, the HydroShear DNA Shearing Device utilizes a precision-drilled ruby as its shearing orifice.

The ruby is mounted inside the *orifice box*, which is connected by tubing to the valve system.

(See *4.5.1 Shearing Assembly Components, page 30* for a detailed description of the shearing assembly).

2.1.2 Pump System

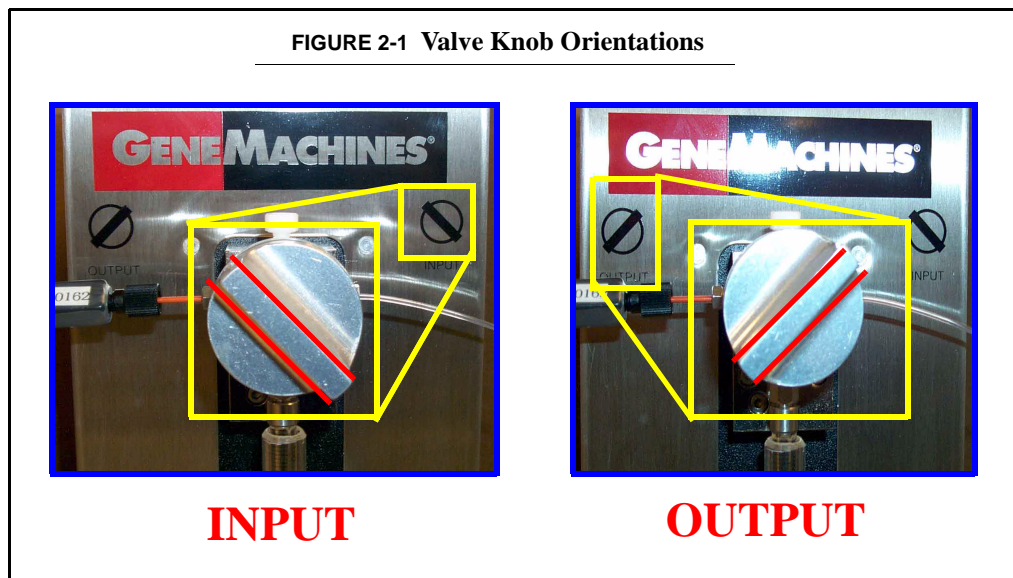
The HydroShear DNA Shearing Device employs a syringe pump system. Air and fluid flow is pressure-controlled by the movement of the plunger inside the glass syringe. The pump will “stall” at a certain pressure; at this pressure, the pump’s functions can be accomplished without promoting leakage throughout the pump and valve system.

1. Oefner, P.J., S.P. Hunicke-Smith, Chiang, F. Dietrich, J. Mulligan, and R.W. Davis. 1996. Efficient random subcloning of DNA sheared in a recirculating point-sink flow system. *Nucleic Acids Res.* **24**: 3879-3886.

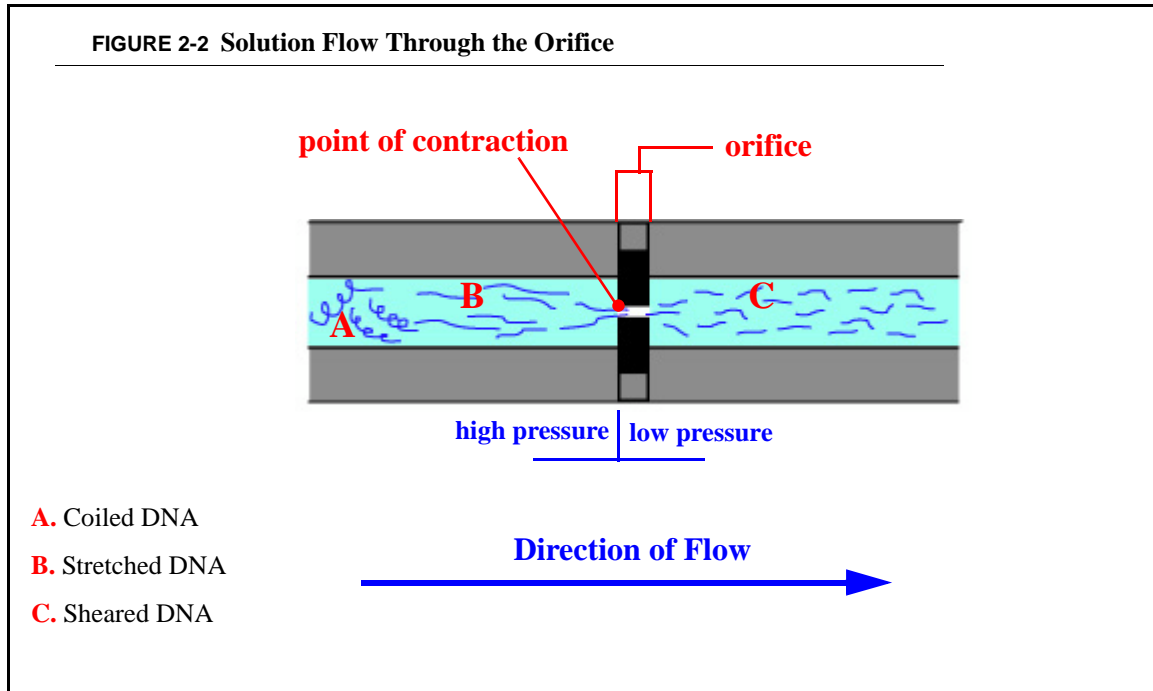
2.1.3 Valve System

The HydroShear DNA Shearing Device utilizes a single, four-port valve. The syringe connects to the lowest port, the output and input tubes attach to the lateral ports, and the top port remains plugged.

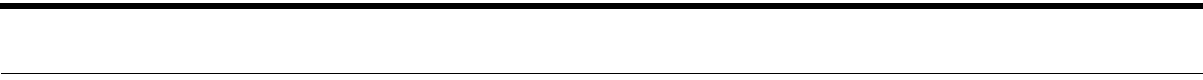
A valve knob mounted above the syringe controls the flow through the lateral valve ports. When the top of the valve knob points to the left, the valve is opened towards the input tube. When the top of the valve knob points to the right, the valve is opened towards the output tube. The valve knob must be manually turned by the user between pump and shearing passes. (FIGURE 2-1)



2.2 SHEARING METHOD



1. The action of the syringe pump plunger forces the DNA in solution towards the orifice. Because the orifice is smaller than the tubing leading to it, the placement of the orifice creates an abrupt contraction in the diameter of the fluid's path.
2. Because the solution is being forced through a smaller opening, the pressure of the solution in the mechanism builds before the orifice and then drops dramatically after passing into the orifice.
3. This dramatic pressure drop, in turn, compels the solution to accelerate in order to maintain its volumetric flow rate. (This acceleration is accordance with Bernoulli's Equation for Frictionless Flow).
4. The acceleration of the solution creates drag forces (i.e. extensional strain forces) that stretch the DNA until its molecular bonds begin to break and the DNA "snaps" into fragments.
5. Fragmenting of the DNA continues until the pieces are too short for the drag forces to break the molecular bonds. The final fragment size (i.e. the length at which the shearing force is too weak to break the bonds) is determined by the flow rate of the fluid and the size of the shearing assembly's orifice.



3. Performance Data

3.1 SUMMARY

- Fragment size is correlated with pump speed and is chosen by the user.
- The HydroShear DNA Shearing Device can completely shear a sample with an initial fragment length as small as 3 kb.
- The HydroShear DNA Shearing Device can fragment a sample volume as small as 40 µl.
- The HydroShear DNA Shearing Device can repeatedly generate a fragment range centered around 1.5 kb if using a standard shearing assembly. Custom shearing assemblies can produce fragment ranges centered around shorter lengths.
- Shearing efficiency is independent of initial fragment length, sample volume, DNA concentration, users, user skill level, and day of

3.2 FRAGMENT SIZE IS CORRELATED WITH PUMP SPEED

The fragment size produced is correlated with the pump speed. The pump speed for a shearing run is designated by the user. (FIGURE 3-1)

Method:

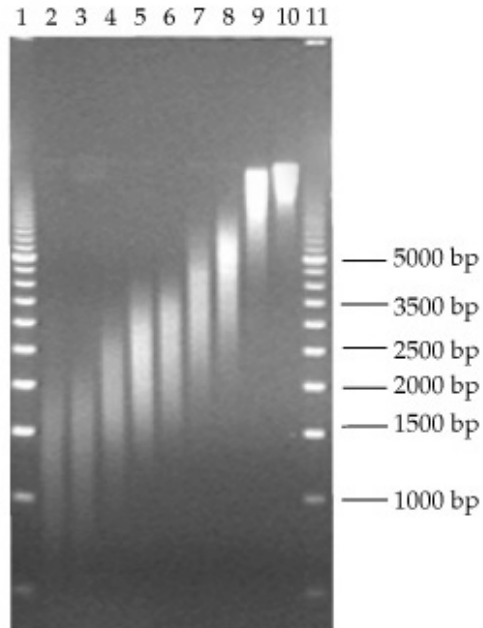
Each of several samples was sheared at a different Speed Code. The sheared samples were run on an agarose gel to determine the resulting fragment sizes.

Result:

As pump speed was increased (i.e. as Speed Code value was decreased), the fragment size produced decreased. Thus, fragment size is inversely correlated with pump speed and is directly correlated with the Speed Code.

FIGURE 3-1 Fragment Size is Correlated with Pump Speed

<u>Lane</u>	<u>Speed Code</u>
2	3
4	5
5	7
6	9
7	11
8	13
9	15
10	17
11	19
1, 12	500 bp ladder



Remember: A lower Speed Code produces a higher pump speed and shorter fragments!

1% agarose gel run at 145V for 2 hours.
All samples are 2 µg/100 µl lambda.

3.3 SAMPLE BOUNDARIES

3.3.1 Smallest Initial Fragment Size that Can Be Sheared

The HydroShear DNA Shearing Device shears nearly all DNA in a sample of 4kb linear fragments. The HydroShear DNA Shearing Device can shear a sample of 3kb linear fragments, but some DNA is left unsheared. (FIGURE 3-2)

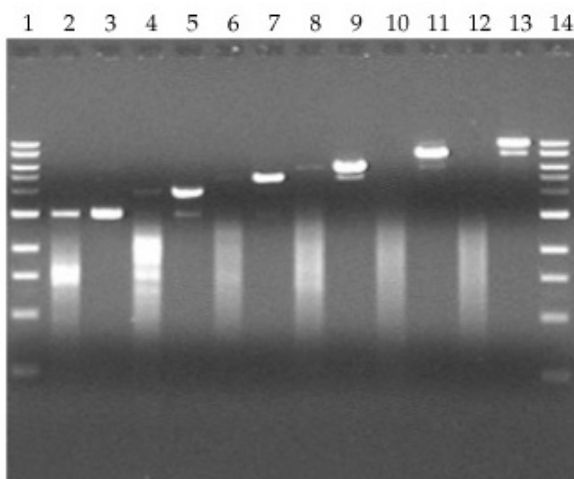
Method:

Samples of varying fragment sizes, ranging from 3 kb to 10 kb were sheared at Speed Code 3. The sheared samples were then run on an agarose gel alongside unsheared samples of corresponding initial fragment sizes.

Result:

At an initial fragment size of 4kb or greater, nearly all of the DNA sample is sheared. Shearing does occur when a sample of 3 kb initial fragment size is run; however, some of the DNA in the sample does remain unsheared.

FIGURE 3-2 Smallest Initial Fragment Size that Shears



1% agarose gel run at 105V for 1 hour.
 All samples gel extracted from a 1kb DNA ladder.
 Sheared about 2ug/100ul. Loaded 0.025ug on gel.
 All samples sheared at speed 3.

<u>Lane</u>	<u>Sample</u>
2	3 kb fragment sheared
3	3 kb sample
4	4 kb fragment sheared
5	4 kb sample
6	5 kb fragment sheared
7	5 kb sample
8	6 kb fragment sheared
9	6 kb sample
10	8 kb fragment sheared
11	8 kb sample
12	10 kb fragment sheared
13	10 kb sample
1, 14	1 kb DNA ladder

3.3.2 Smallest Sample Volume that Can Be Sheared

The HydroShear DNA Shearing Device can shear a sample volume as small as 40 µl. (FIGURE 3-3)

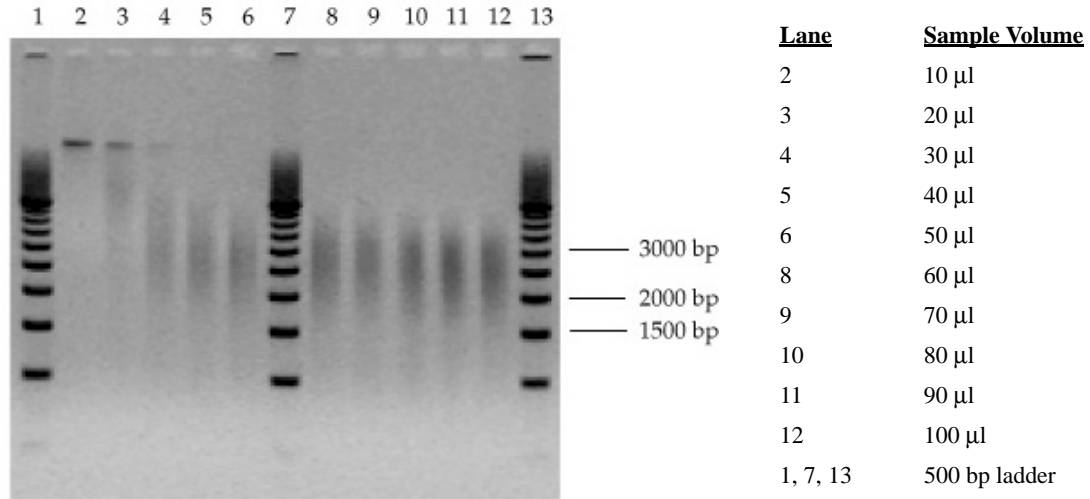
Method:

Samples of varied volumes ranging from 10µl to 100µl were sheared at Speed Code 10. The sheared samples were then run on an agarose gel.

Result:

Sample volumes of 40µl or greater resulted in a consistent fragment length range.

FIGURE 3-3 Smallest Volume that Can Be Sheared



1% agarose gel. Run at 105 V for 1 hour.
All samples are 0.01ug/ μ l lambda DNA sheared at speed 10.

3.4 SMALLEST FRAGMENTS THE HYDROSHEAR DNA SHEARING DEVICE CAN REPEATEDLY PRODUCE

The HydroShear DNA Shearing Device can repeatedly generate fragments centered at 1 kb. (FIGURE 3-4)

However, GeneMachines' biologists continue to conduct experiments and have shown promising results about the HydroShear DNA Shearing Device's ability to produce even smaller fragments. Please contact GeneMachines for the latest results. (See 1.4 Contacting GeneMachines®, page 10)

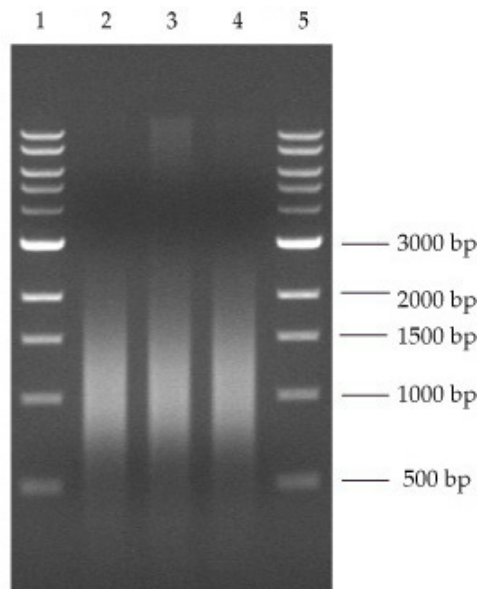
Method:

Three samples with a 50 kb initial fragment length were sheared, using a custom shearing assembly, at a Speed Code of 3. (The standard shearing assembly produces 1.5 kb fragments). The resulting fragments were then run on an agarose gel.

Result:

All three samples results in a fragment size range of 750 bp to 1.5 bp. The HydroShear DNA Shearing Device can repeatedly generate fragments centered at 1 kb.

FIGURE 3-4 Smallest Consistently-Produced Fragment Range



1% agarose gel run at 105V for 1 hour.
All samples taken from the same stock of 0.02ug/ul of lambda DNA. Sheared 50 µl each sample.

3.5 INDEPENDENCE OF SHEARING PERFORMANCE

GeneMachines' biologists have determined that the size distribution produced is affected by:

1. **The geometry of the shearing assembly**
2. **The flow rate**

(See 3.2 Fragment Size is Correlated with Pump Speed, page 17)

When the shearing assembly geometry and the flow rate are kept consistent, the size distribution of the DNA fragments produced is highly reproducible.

3.5.1 No Effect of Initial Fragment Length

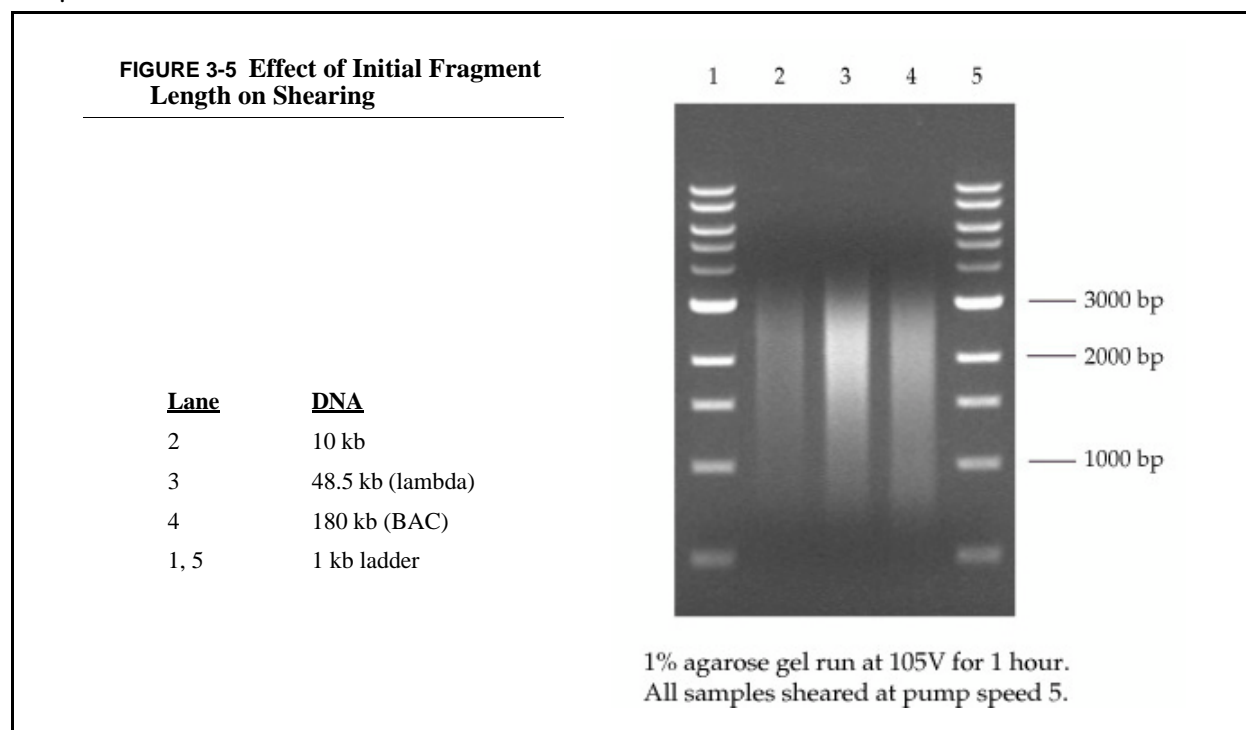
Shearing results are independent of initial fragment length. (FIGURE 3-5)

Method:

Samples of varying lengths were sheared at Speed Code 5.

Result:

Each sample resulted in sheared fragments of identical lengths. Thus, the initial fragment length of the sample does not affect the post-shearing fragment length.



3.5.2 No Sample Volume Effect

Shearing results are independent of sample volume. (FIGURE 3-6)

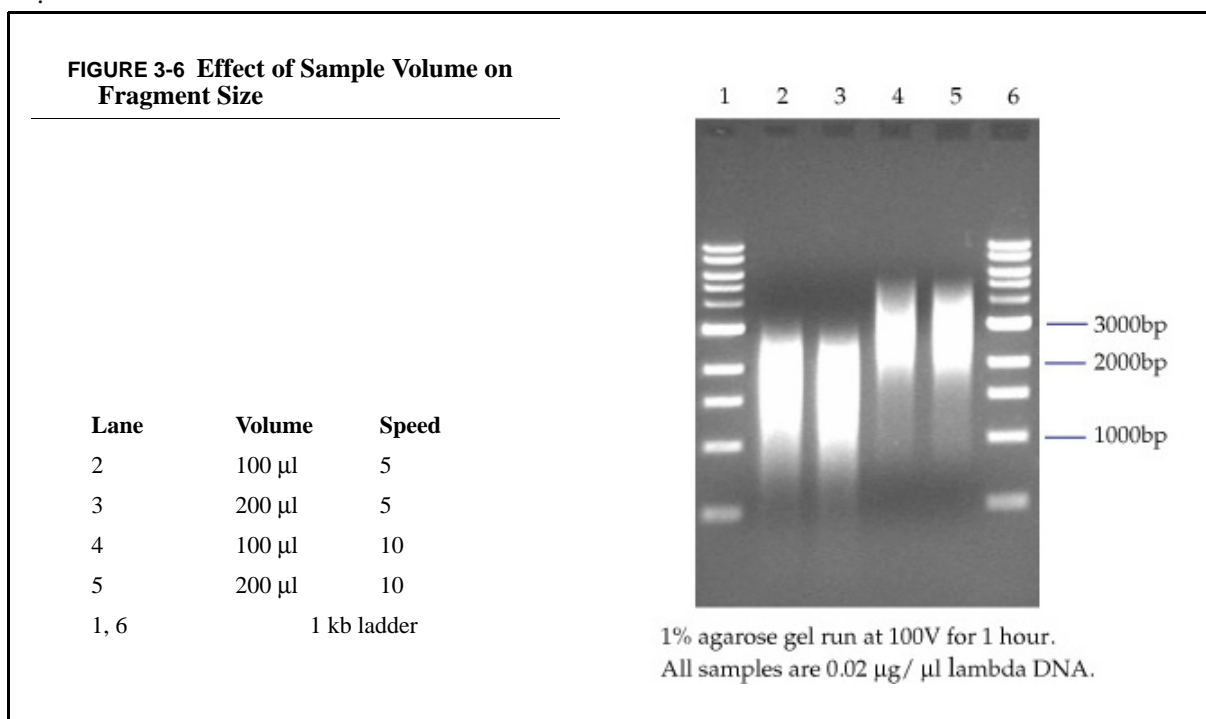
Method:

Performance Data

A 100 μ l sample and a 200 μ l of lambda DNA were sheared at Speed Code 5. A 100 μ l sample and a 200 μ l sample of lambda DNA were sheared at Speed Code 10. After shearing, 0.2 μ g of all four samples were run on a 1% agarose gel at 100V for 1 hour.

Result:

Samples of different volumes resulted in identical fragment lengths when sheared at the same Speed Code. Thus, shearing is not affected by sample volume.



3.5.3 No DNA Concentration Effect

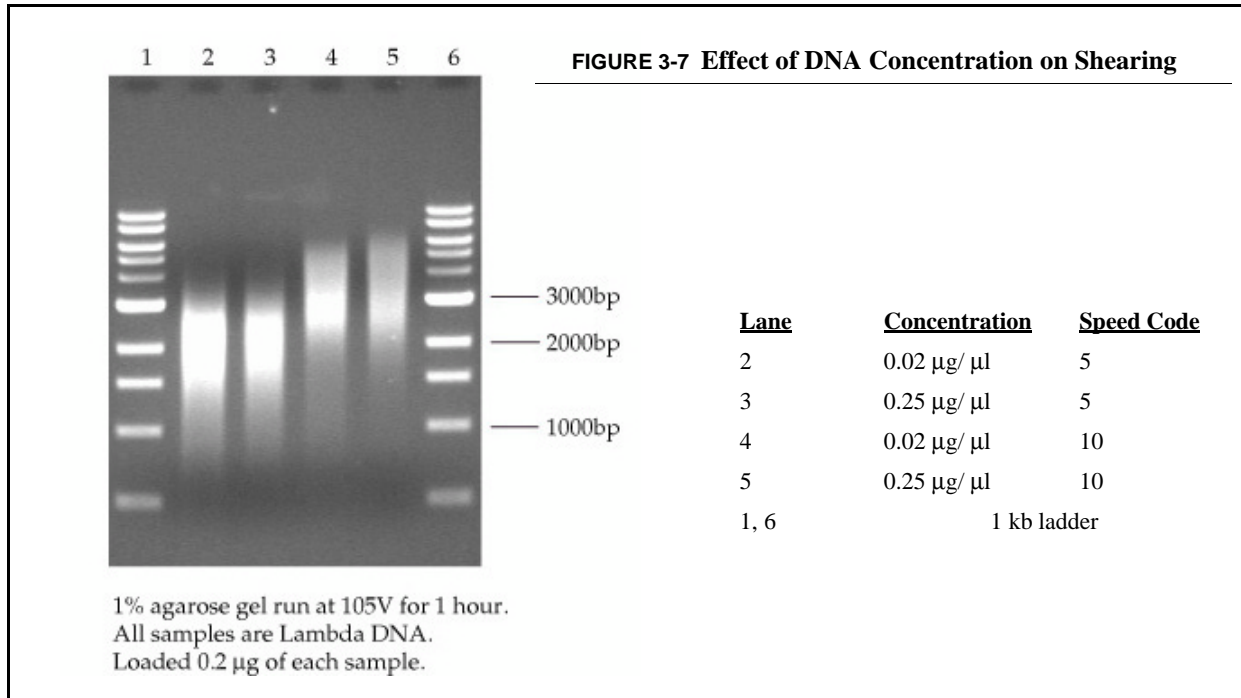
Shearing results are independent of DNA concentration. (FIGURE 3-7)

Method:

Two samples of lambda DNA with a concentration 0.02 μ g/ μ l were sheared at Speed Code 5. Two samples of Lambda DNA with a concentration of 0.25 μ g/ μ l were sheared at Speed Code 10. After shearing, 0.2 μ g of all four samples were run on a 1% agarose gel at 105V for 1 hour.

Result:

Samples of different concentrations resulted in identical fragment lengths when sheared at the same Speed Code. Thus, the concentration of the DNA samples did not affect the shearing results.



3.5.4 No User, User Skill, or Sequence Effect

Shearing results are independent of the user, the user's skill, and the sequence of shearing runs. (FIGURE 3-8)

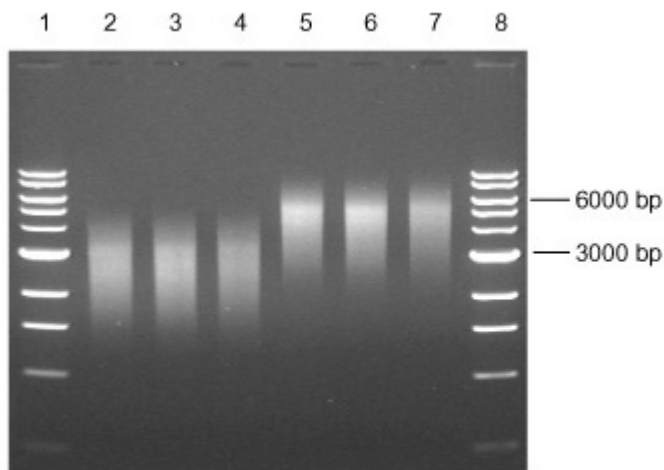
Method:

Six samples were taken from the same stock of lambda DNA. On the same day, a beginning user and an advanced user each sheared a sample at Speed Code 10. Also on this day, a beginning user and an advanced user each sheared a sample at Speed Code 14. On a second day, an intermediate user sheared one sample at Speed Code 10 and a second sample at Speed Code 14.

Result:

Samples sheared at the same Speed Code resulted in identical fragment sizes, regardless of the user, the skill of the user, or the day of shearing. Thus, neither the user, nor the skill of the user, nor the sequence of shearing runs affect the shearing results.

FIGURE 3-8 Consistency of Shearing Across Users and Days



1% agarose gel run at 100V for 1 hour.
 All samples taken from same stock of lambda DNA.
 Sheared 2 µg/100 µl. Loaded 0.2 µg of each sample.

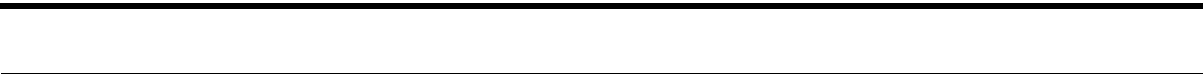
Lane	Pump Speed	Skill Level*	Day
2	10	Experienced	B
3	10	Intermediate	A
4	10	Beginner	B
5	14	Experienced	B
6	14	Intermediate	A
7	14	Beginner	B
1, 8		1 kb ladder	

***Skill Levels**

Experienced: has used machine for over two years

Intermediate: has used machine for two months

Beginner: has never used machine



4. Installing the HydroShear DNA Shearing Device

4.1 FINDING A LOCATION FOR THE HYDROSHEAR DNA SHEARING DEVICE

Find a location for the HydroShear DNA Shearing Device that meets the following guidelines:

1. A properly grounded 110V/15amp wall outlet should be within reach of the HydroShear DNA Shearing Device's power cord.
2. Ideally, the HydroShear DNA Shearing Device and the controlling CPU's monitor should be placed in full view of each other.
3. Choose a location away from any vents that could expel particulate material on the machine.
4. Ensure that the location satisfies the HydroShear DNA Shearing Device's environmental requirements. (See 1.3.3 Environmental Requirements, page 10)

4.2 SYRINGE INSTALLATION

NOTE: It is easiest to install the syringe before installing the hardware so that the pump plunger is lowered enough to allow for attachment of the syringe. **If the pump's plunger is fully raised**, you will need to complete 4.3 Hardware Installation, page 29, and 4.4 Software Installation, page 29, before installing the syringe. Then, use the Manual Operation Window to lower the plunger by ejecting 250 μ L of air from the pump. (See 7.3 Ejecting Fluid from the Pump, page 54).



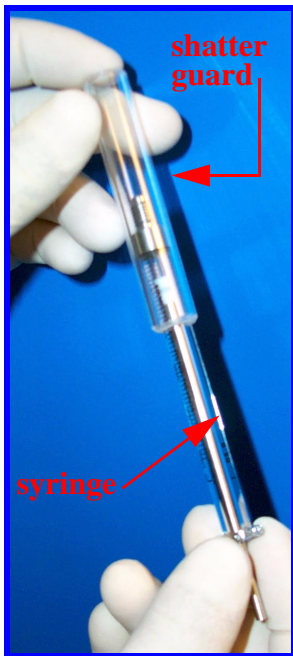
- Wear safety goggles when operating the HydroShear DNA Shearing Device.
- Take care when working with the syringe; it is made of glass and could shatter under pressure!
- When maneuvering the syringe, always grasp the metal ring at the top of the syringe tip.

Follow these steps to install the syringe:

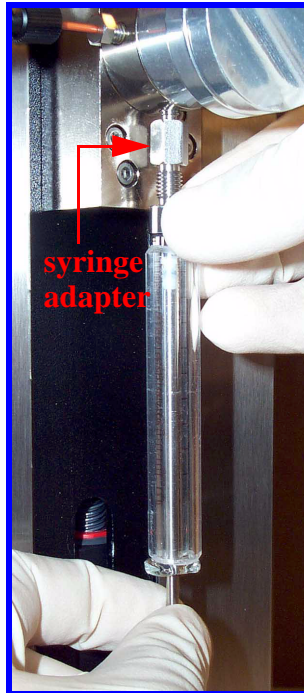
1. **Place the plastic shatter guard over the syringe and plunger assembly. (FIGURE 4-1)**
2. **Screw the syringe (counterclockwise) into the syringe adapter.**
The syringe adapter is the hex bolt mounted underneath the valve. You may need to shorten the assembly first so that the syringe needle doesn't run into the syringe mount. If this is the case, gently guide the needle farther up into the syringe.
3. **Gently pull down on the plunger and guide the plunger's tip into the small hole in the center of the syringe mount. (FIGURE 4-1)**
The syringe mount is the metal rectangle attached to the metal shaft that extends laterally from the pump casing.
4. **Using the 0.050" Allen wrench, tighten the set screw on the side of the syringe mount. (FIGURE 4-1)**

FIGURE 4-1 Installing the Syringe

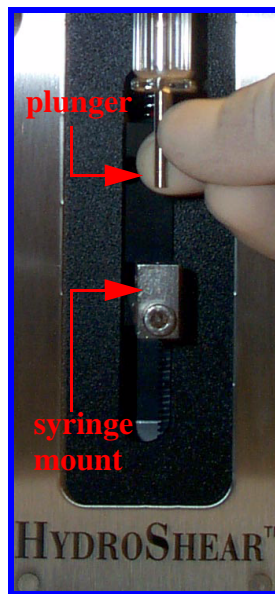
Putting plastic shatter guard on syringe. (Step 1)



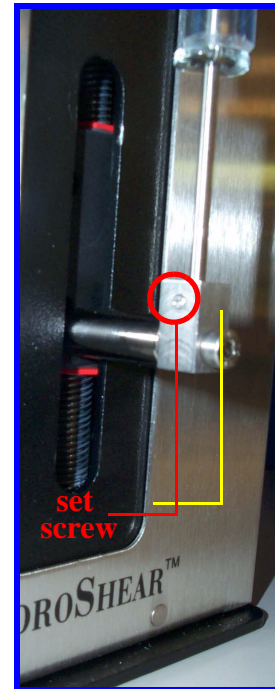
Screw syringe into syringe adapter. (Step 2)



Pulling plunger down into syringe mount. (Step 3)

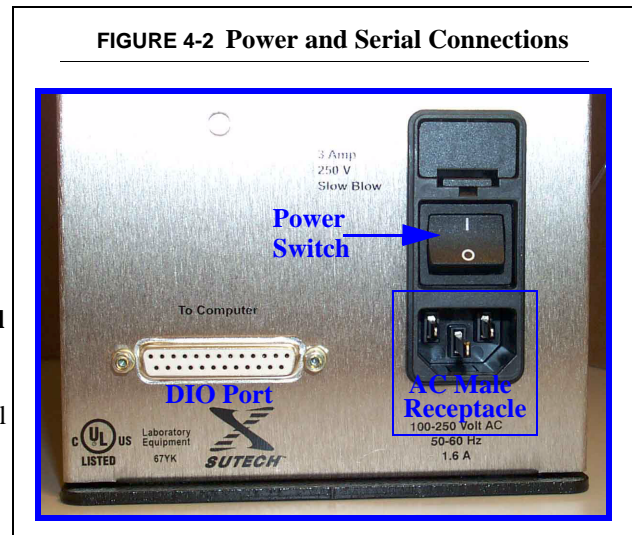


Set screw on syringe mount. (Step 4)



4.3 HARDWARE INSTALLATION

1. Plug the serial cable's 25-pin DIO connector into the DIO port, located at the lower left of the HydroShear DNA Shearing Device's back panel. (FIGURE 4-2)
2. Secure the DIO connection to the HydroShear DNA Shearing Device by tightening the screws on either side of the DIO connector.
3. Plug the serial cable's serial connector into a serial port on the CPU. (FIGURE 4-2)
After installing the HydroShear DNA Shearing Device software, you will need to specify which serial port is used in the software's Machine Parameters Entry Window. (See 5.6.1.5 Port Number, page 43).



4. Plug the power cord's AC connector end into the AC male receptacle on the back of the HydroShear DNA Shearing Device. (FIGURE 4-2)
5. Plug the power cord's three-prong end into a properly grounded wall outlet (110V / 15amp).
6. Turn the HydroShear DNA Shearing Device's power switch into the On position.
The power switch is located on the back of the machine, at the lower right corner (as viewed from the back).
7. After installing the HydroShear DNA Shearing Device software (See 4.4 Software Installation, page 29), open the software by locating the program titled "HydroShear DNA Shearing Device" in the Windows Start ⇒ Programs menu.

4.4 SOFTWARE INSTALLATION

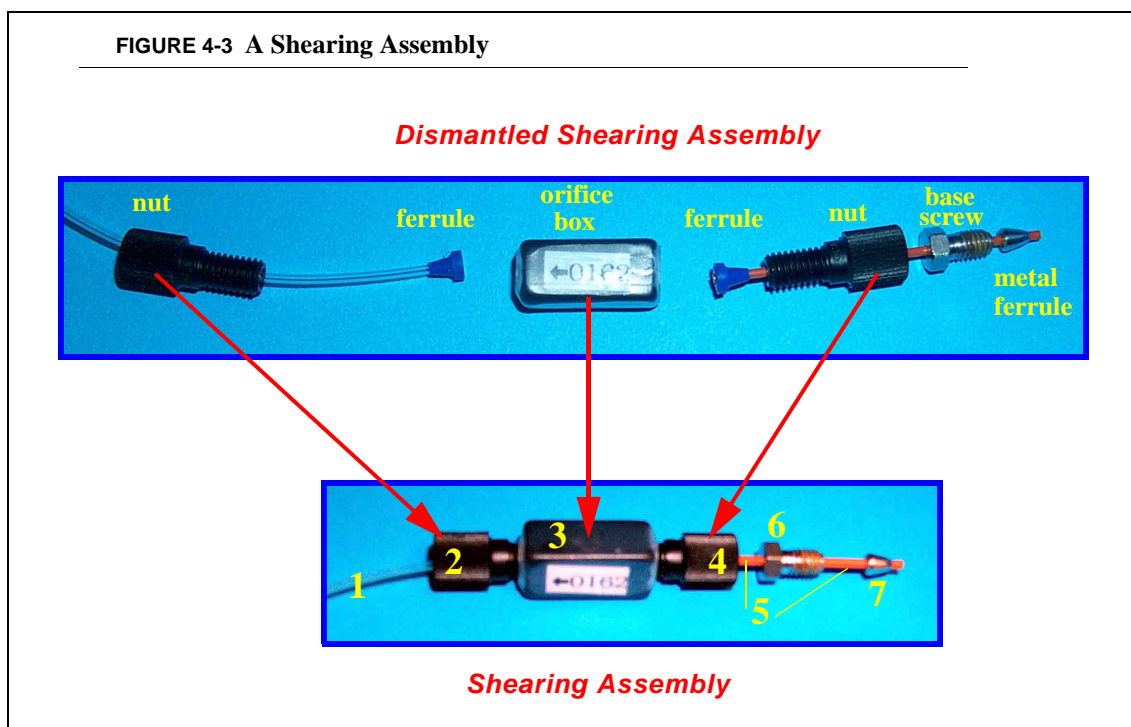
1. Insert the HydroShear DNA Shearing Device installation disk into the CPU's floppy disk drive.
2. Run the file "setup.exe" from the installation disk.
On most PC's, this can be accomplished by following these steps:
 1. Select "Run. . ." from the Start menu.
--A dialog box appears prompting you to specify the file to run.
 2. Type "A:\Setup.EXE" in the text box.
 3. Click "OK."
--The Install software program opens.
--Follow the instructions displayed onscreen by the Install software.

4.5 SHEARING ASSEMBLY INSTALLATION



The shearing assembly must be oriented correctly, both to prevent irreversible damage to the orifice and to function properly. Carefully read the instructions below and refer to Figure 4-3, “A Shearing Assembly,” on page 30 to ensure that you correctly install the shearing assembly.

4.5.1 Shearing Assembly Components



The components of the shearing assembly (FIGURE 4-3) must be correctly ordered and oriented in order to function properly and prevent damage to the machine. Although the shearing assembly is always correctly assembled at GeneMachines, you may use the following guidelines to ensure that the components are correctly ordered and oriented before attaching the assembly to the machine:

Starting at the clear plastic tubing and moving from left to right, the following components of the shearing assembly should be visible (FIGURE 4-3):

1. Clear plastic tubing

This tube serves as the *output tube*. The sheared samples and used wash solution will be dispensed from this tube. If this tube is detached from the shearing assembly (by unscrewing the tubing nut), you can see a plastic ferrule attached to the end that is inserted into the shearing orifice. The ferrule is oriented so that the untapered end is flush with the end of the tubing.

2. Tubing nut

This nut screws into the orifice box at the head of the orifice box's arrow label. It attaches the output tube to the shearing assembly.

3. Orifice box

The orifice box of any shearing assembly shipped after March 2000 is labeled with an arrow and an identification number. The arrow should always point towards the clear plastic output tubing. If the arrow is missing, you can observe the inside of the orifice to determine the correct orientation. (*See 4.5.2 Determining the Correct Orientation of an Unlabeled Orifice Box, page 31*)

The identification number is useful for keeping calibration records.

4. Tubing nut

This nut screws into the orifice box at the tail of the orifice box's arrow label. It attaches the orifice box to the PEEK tubing (the orange inflexible tubing) leading to the base screw.

5. Peek tubing

This orange, inflexible tubing is attached to the orifice box via the tubing nut (4) and is attached to the base unit via the base screw (6).

6. Base screw

Called the "base screw" since it attaches the shearing assembly to the base unit, this screw is a 1/16" hex screw. The threaded side of the base screw should be closest to the metal ferrule.

7. Metal ferrule

The tapered end of this ferrule should be closest to the end of the orange tubing.

4.5.2 Determining the Correct Orientation of an Unlabeled Orifice Box

If you have an orifice box without an arrow label, follow these steps to determine the correct orientation:

1. Remove all tubing from the orifice box and hold it near a light.

2. Look through the tubing nut ports to observe a pinkish circle of color; this is the ruby.

One side of the ruby will appear smaller and less reflective than the other.

3. Screw the output tube's tubing nut into the port through which the ruby appears smaller and less reflective.

4. Screw the tubing nut leading to the orange inflexible tubing and base screw into the port through which the ruby appears larger and more reflective.

4.5.3 Attaching the Shearing Assembly to the HydroShear DNA Shearing Device

1. Check to see that the three components of the shearing assembly are correctly oriented. (See 4.5.1 Shearing Assembly Components, page 30)
2. Twist the base screw into the port on the left side of the valve.
The left side of the valve is the output side. Because the shearing assembly leads to the output tube, the shearing assembly's base screw port is located on this left, output side.
3. Tighten the base screw connection with the 1/4" hex wrench.
4. Calibrate the shearing assembly (if new or if your calibration records have been misplaced).
Each new shearing assembly needs to be individually calibrated. (See 4.5.4 Calibrating the Shearing Assembly, page 33).

FIGURE 4-4 Attaching the Shearing Assembly to the Base Unit

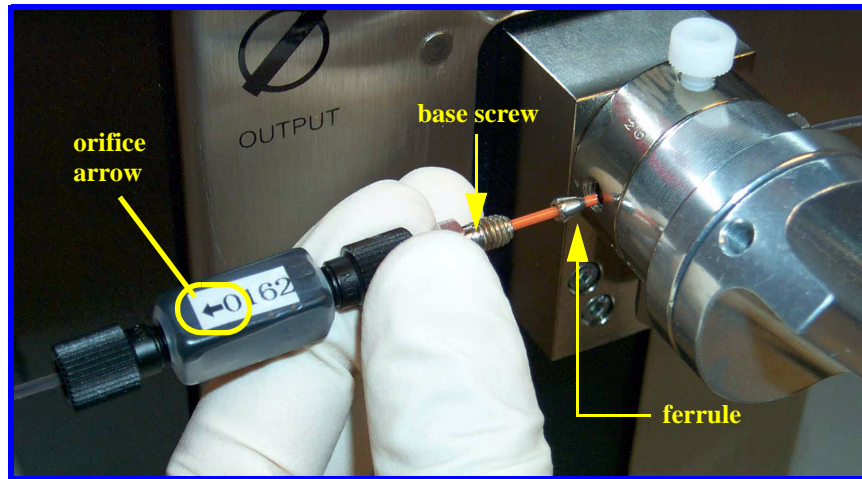
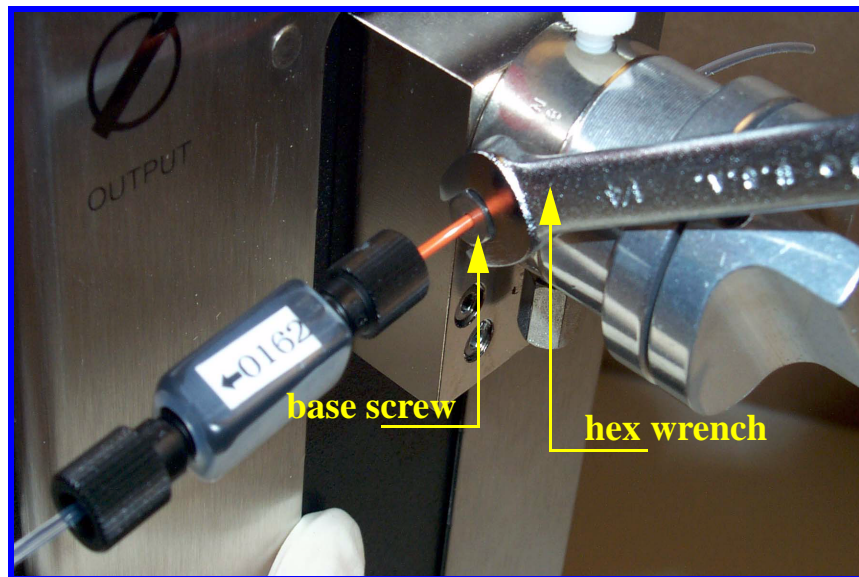


FIGURE 4-5 Tightening the Base Screw



4.5.4 Calibrating the Shearing Assembly

Each new shearing assembly needs to be individually calibrated. Since the size of each shearing assembly's orifice is slightly different, the same Speed Code, when used with different shearing assemblies, will not necessarily result in the same fragment size. The typical variation in fragment size produced by different shearing assemblies is approximately 500 bp. This calibration will determine the Speed Code to fragment size correlation for a specific shearing assembly.

To calibrate a shearing assembly, follow these steps:

- 1. Shear several DNA samples at various Speed Code values, keeping the Volume, the Number of Cycles, and the DNA concentration constant.**

Shearing at four different Speed Codes should be sufficient for most users. To increase the precision of the calibration, shear at more Speed Codes.

- 2. Compare the resultant fragment sizes by gel electrophoresis, keeping track of which Speed Codes produced which fragments.**

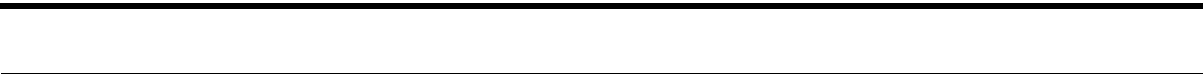
- 3. Make a record of the average fragment size produced at each Speed Code.**

Use the identification number on the shearing assembly's orifice box.

You can then reference this record when determining the Speed Code for future shearing runs.

TABLE 1. Typical Size to Speed Code Correlation

Speed Code	Average Fragment Size (kb)
3	1-2
9	1.5-3
15	5-10



5. Software

5.1 STARTING THE HYDRO SHEAR DNA SHEARING DEVICE SOFTWARE

1. **To start the HydroShear DNA Shearing Device software, locate the program titled “HydroShear DNA Shearing Device” in the Windows Start ⇒ Programs menu.**

The HydroShear DNA Shearing Device Main Panel appears, and the pump is initialized.

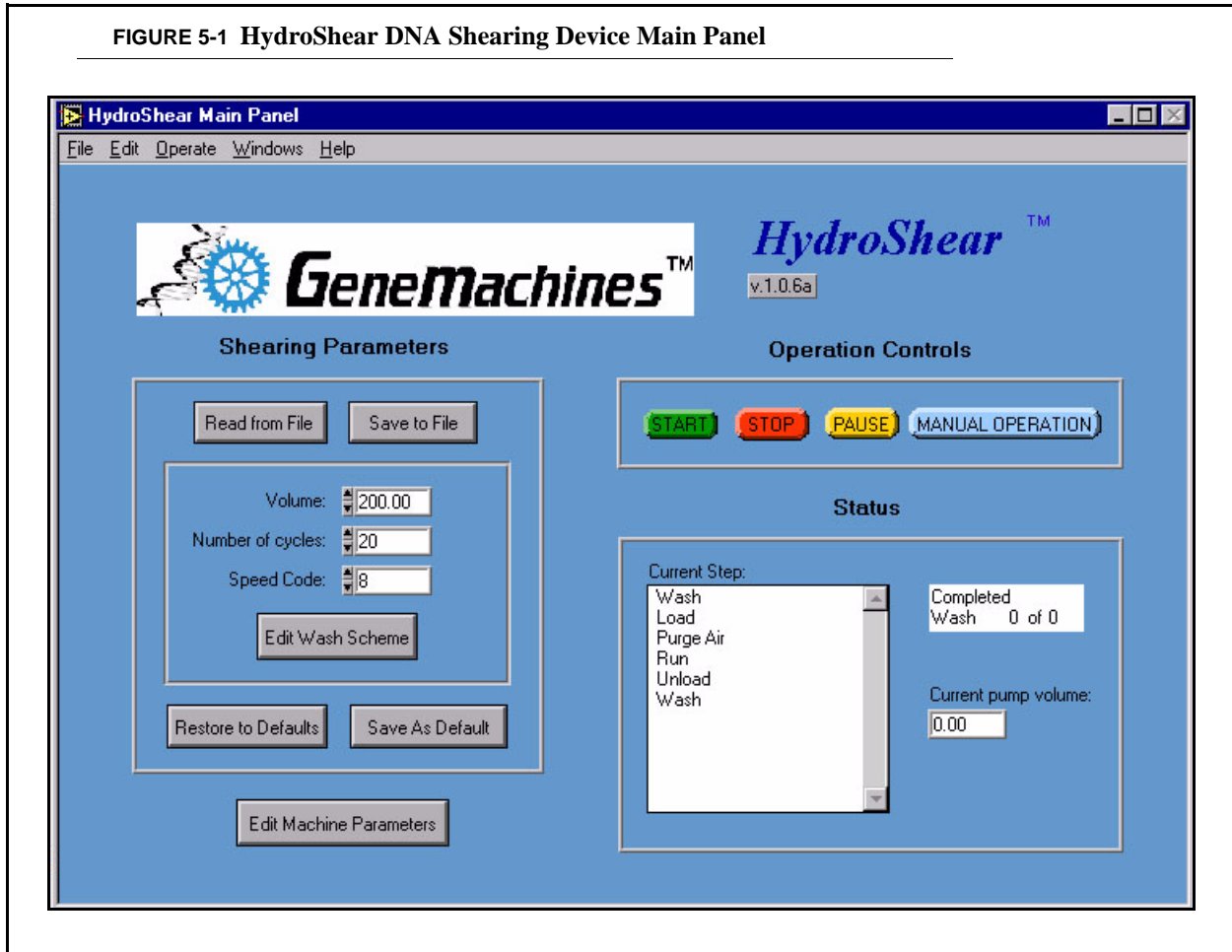
A dialog box appears displaying the following message: “Machine parameters are currently set to the factory defaults. If you don’t wish to use factory settings, you should specify other values for the machine before continuing.”

2. **Click “OK.”**

The dialog box disappears.

Continue by resetting machine parameters (*See 8. Machine Parameters, page 51*), setting shearing parameters (*See 5.3 Shearing Parameters, page 37*), or shearing (*See 6. Shearing, page 45*).

FIGURE 5-1 HydroShear DNA Shearing Device Main Panel



5.2 A TOUR OF THE HYDROSHEAR DNA SHEARING DEVICE MAIN PANEL

The HydroShear DNA Shearing Device Main Panel is the HydroShear DNA Shearing Device’s control and feedback center. The HydroShear DNA Shearing Device Main Panel is divided into three boxed areas titled “Shearing Parameters,” “Operation Controls,” and “Status.”

Shearing Parameters

You will use this box to customize shearing parameters for the upcoming shearing run, save customized shearing parameters to a file, instruct the software to use parameters saved to a file, or set new default parameter values. For detailed explanations and instructions, see *5.3 Shearing Parameters, page 37*.

Operation Controls

This box includes the buttons used to run the HydroShear DNA Shearing Device and a button used to navigate to the Manual Operation window. (See 7. Manual Operation, page 53)

For detailed explanations of the Operation Controls and instructions for use, see 5.4 Operation Controls, page 40.

Status

This box displays the status of a current shearing run or wash, as well as the current pump volume. For detailed explanations and instructions, see 5.5 Status, page 41.

5.3 SHEARING PARAMETERS

The shearing parameters, including the basic shearing parameters and the wash scheme, can be edited using the Shearing Parameters box, located on the left side of the HydroShear DNA Shearing Device Main Panel.

5.3.1 Basic Shearing Parameters Defined

Three basic shearing parameters are referenced by the HydroShear DNA Shearing Device software: Volume, Number of Cycles, and Speed Code. The default value for each is displayed upon starting the software. However, these values can be adjusted for each run (See 5.3.2 Editing Basic Shearing Parameter Values, page 38), or new defaults can be defined (See 5.3.5 Saving New Shearing Parameter Default Values, page 39):

5.3.1.1 Volume

This is the volume of the sample, in microliters (μL) you are preparing to shear. This value can range from the Void Volume to the “Syringe Volume,” both of which are specified in the Machine Parameters window. (See 8.1 The Machine Parameters defined, page 51). However, GeneMachines’ biologists have found that shearing will be incomplete unless the Volume is at least 40 μL . (See 3.3.2 Smallest Sample Volume that Can Be Sheared, page 19).



If your sample volume is greater than 300 μL , you will need to use the longer output tube included with your HydroShear DNA Shearing Device.

5.3.1.2 Number of Cycles

This number refers to the number of shearing passes you want the machine to perform on the sample. A typical Number of Cycles is 20; after the twentieth cycle, additional shearing passes are unlikely to shear the sample any further.

5.3.1.3 Speed Code

The Speed Code correlates with the speed of the pump when performing the shearing passes. The optimal value depends on both the individual orifice size of the shearing assembly (there are slight variations in size among orifices) and the fragment size desired.

The Speed Code can range from 0 to 40, with an increasing Speed Code value corresponding with a decreased pump plunger speed. A Speed Code of 0 results in the fastest speed (1.2 seconds per stroke), while a Speed Code of 40 results in the slowest speed (600 seconds per stroke).

Note that, if the Speed Code entered is less than 20, the software will automatically insert three “pre-passes” into the shearing run. These pre-passes will occur before the actual shearing cycles and are executed at gradually higher speeds to prepare for the high-speed shearing passes.



Standard-size shearing assemblies normally should not be operated at a Speed Code lower than 3; standard-sized shearing assemblies cannot perform at the lowest Speed Codes (0,1, and 2) and will produce a pump overload message.

Remember! The lower the Speed Code, the faster the speed!

5.3.2 Editing Basic Shearing Parameter Values

To edit a parameter value, follow these steps:

1. **Locate the text box next to the parameter name (e.g. “Volume”).**
2. **Either click in the text box and type in the desired value, or click the arrows next to the text box to display the desired value.**

NOTE: Should you edit basic shearing parameter values and/or wash scheme values and wish to restore the default values, simply click the **RESTORE TO DEFAULTS** button, located at the bottom left corner of the Shearing Parameters box on the HydroShear DNA Shearing Device Main Panel. Upon clicking this button, the last set of basic parameter values that were saved as default values will be displayed in the text boxes. Remember that this will restore both the default basic shearing parameter values and the default wash scheme values!

5.3.3 The Wash Scheme

The wash scheme should be conducted before and after each shearing run to clean any residual sample solution from the machine. The wash scheme is defined in, and can be edited in, the Edit Wash Scheme window.

To reach the Edit Wash Scheme window, click the **EDIT WASH SCHEME** button on the HydroShear DNA Shearing Device Main Panel.

To define a wash scheme, follow these steps:

1. **Click in the top text box in the column headed “Solution.”**
2. **Type a name for the first wash solution.**
3. **In the first text box underneath the heading “Cycles,” specify the number of wash cycles that should be completed using the first wash solution. To do so, either click in the text box and type a number, or click the arrows next to the text box to change the displayed number.**
4. **Repeat Steps 1-3, using the second text box in each column for the second wash solution and the third for the third wash solution.**
5. **Click the OK button, located at the bottom of the Edit Wash Scheme window.**
The Edit Wash Scheme window disappears. The HydroShear DNA Shearing Device Main Panel Appears.

Typical Wash Scheme

A typical wash scheme consists of:

1. HCl -- 4 cycles
2. NaOH -- 4 cycles
3. Water/Buffer -- 4 cycles

5.3.4 Saving Shearing Parameters Values to a File

Custom shearing parameter values can be saved to a file, allowing for easy recall and entry when conducting future shearing runs. This file is user-named and will be stored in the user-specified location.

To save shearing parameter values to a file, follow these steps:

1. **After entering values for each basic shearing parameter (See 5.3.2 *Editing Basic Shearing Parameter Values*, page 38) and for the wash scheme (See 5.3.3 *The Wash Scheme*, page 38), click the SAVE TO FILE button.**
The “Choose file to write. . . “ dialog box appears.
2. **Navigate to the location where you would like to save the file.**
3. **Type a name for the file in the text box.**
4. **Click the SAVE button.**
The new shearing parameters values are saved with the specified name in the specified location.

5.3.5 Saving New Shearing Parameter Default Values

1. **After entering values for each basic shearing parameter and for the wash scheme (See 5.3.2 *Editing Basic Shearing Parameter Values*, page 38 and 5.3.3 *The Wash Scheme*, page 38), click the SAVE AS DEFAULT button.**
The new values are saved and are used as the default values for every shearing run.



Remember that the shearing parameter default values include default wash scheme values; clicking the **SAVE AS DEFAULT** button will save both the currently entered basic parameter values and the currently entered wash scheme values as the new default values!

5.3.6 Restoring Shearing Parameter Default Values

Should you edit the shearing parameter values (i.e. the basic shearing parameter values and/or wash scheme values) and wish to restore the default values:

1. Click the **RESTORE TO DEFAULTS** button, located at the bottom left corner of the Shearing Parameters box on the HydroShear DNA Shearing Device Main Panel.

The most recently saved default values are displayed in the text boxes.

Note that this will restore both the default basic shearing parameter values and the default wash scheme values!

5.4 OPERATION CONTROLS

Four buttons are located in the Operation Controls box on the HydroShear DNA Shearing Device Main Panel:



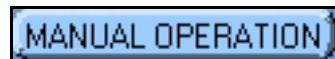
Click the **START** button to start a shearing run. (See 6. Shearing, page 45).



Click the **STOP** button to stop a shearing run already in progress. The machine will stop after the current pump pass is complete.



Click the **PAUSE** button to pause the current shearing run. (The machine will complete its current shearing pass and then pause). While paused, click this button again to resume the shearing run. Take care not to repeatedly click this button to “speed up” the software and/or machine’s response; each click is registered and acted upon. Thus, repeated clicking will start a cycle of pause/unpause/pause/unpause and so on.



Click the **MANUAL OPERATION** button to display the Manual Operation window, where you can conduct individual shearing steps. (See 7. Manual Operation, page 53).

5.5 STATUS

The Status box displays three status items -- Current Step, Wash Status, and Current Pump Volume -- and can be particularly useful when diagnosing and resolving troubleshooting issues. (See 9. Troubleshooting, page 67). The status items are described below:

Current Step

This box displays a list of each step of the current shearing run. When a shearing run is in progress, the current step is highlighted.

Progress

The white box in the top right corner of the Status box displays the progress of the current step (highlighted in the Current Step box). Progress is displayed in the following format: <number of cycles completed> of <total number of cycles in step>.

Note that, when viewing the progress of a Wash step, the total number of wash cycles is the total of all wash cycles specified to occur with each of the three wash solutions. For example, if you specified in your wash scheme that you would like to conduct four wash cycles with each of the three wash solutions, the total number of wash cycles is twelve. (See 5.3.3 The Wash Scheme, page 38).

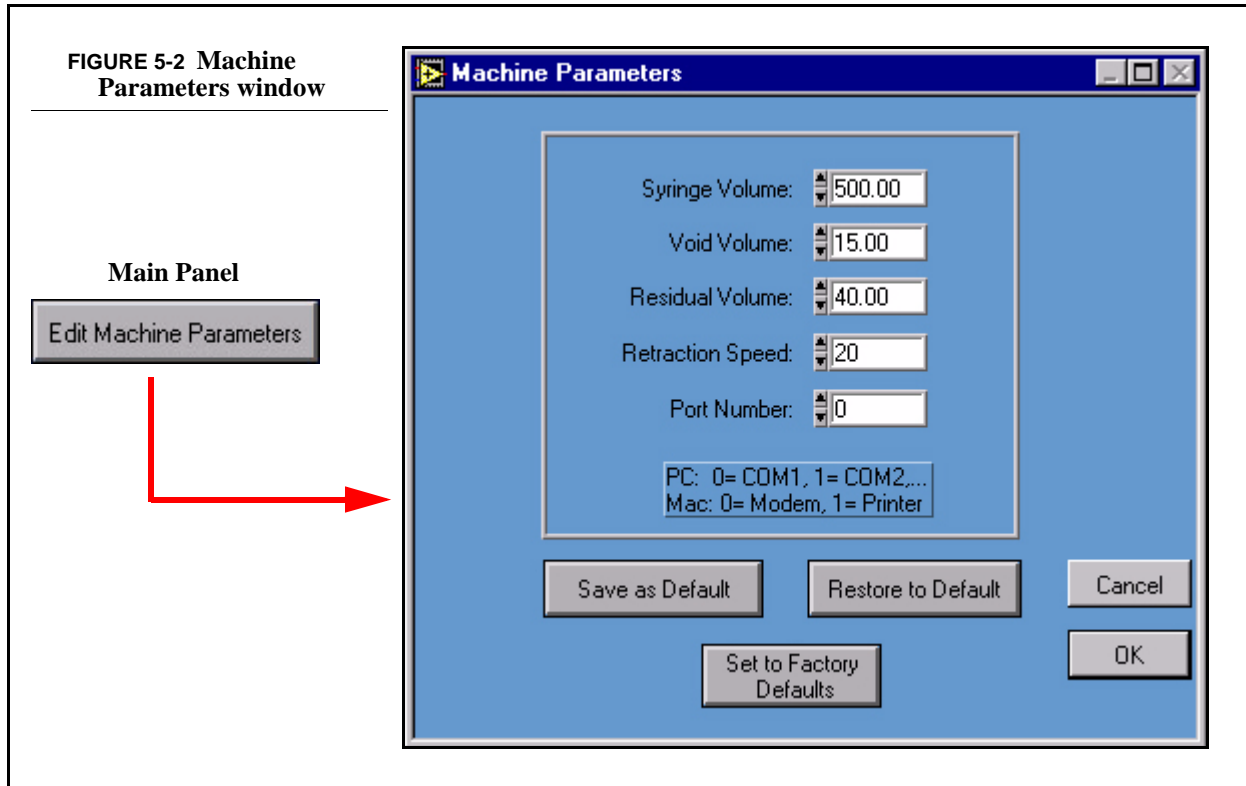
Current Pump Volume

This box displays the volume of liquid (in μL) currently in the syringe.

5.6 MACHINE PARAMETERS

Several machine parameters that are determined and set at GeneMachines can be adjusted in the **Machine Parameters window**. New default values can be specified, or the saved default values or factory-set values can be restored. It is extremely unlikely that these parameter values will require user adjustment.

To open the Machine Parameters window, click the **EDIT MACHINE PARAMETERS** button on the HydroShear DNA Shearing Device Main Panel.



5.6.1 The Machine Parameters Defined

5.6.1.1 Syringe Volume

This value is the volume of the syringe, in μL , that you have installed on your HydroShear DNA Shearing Device. The volume of the syringe included with the HydroShear DNA Shearing Device is $500\mu\text{L}$. The device can also accommodate 1.0 mL, 2.5 mL, 5.0 mL, and 10.0 mL syringes, although the maximum shearing speed decreases as the syringe size increases.

5.6.1.2 Void Volume

This value is the volume of fluid that must remain in the orifice during shearing. The accuracy of this value is imperative for proper shearing. If the volume entered is too large, some DNA will be left unsheared because the fluid will not be retracted far enough into the syringe. If the volume entered is too small, fluid will be retracted too far past the orifice, creating a jet effect; DNA will move through the orifice so quickly that the resultant fragments will be shorter than the Speed Code would indicate. The Void Volume is normally set to $15\mu\text{L}$.

5.6.1.3 Retraction Speed

This value refers to the speed of the pump during the retraction pass and corresponds with the number of seconds required to complete one pump pass.

The Retraction Speed must be kept slow relative to the Speed Code used to shear samples. This prevents the creation of air bubbles in the sample and prevents shearing from occurring during the retraction pass.

The Retraction Speed value ranges from 0 to 40, with 0 corresponding to a stroke speed of 1.2 seconds per stroke and 40 corresponding to a stroke speed of 600 seconds per stroke. Thus the higher the Retraction Speed value, the lower the speed. 20 is a reasonable Retraction Speed value for a normal size shearing orifice; the Retraction Speed value should be higher for smaller orifices.

5.6.1.4 Residual Volume

The definition and value of the Residual Volume parameter differs according to the software version used:

V1.06: The Residual Volume is the volume of air bubbles that may occur in the syringe. This value is referenced by the software during its automatic bubble clearing routine and should be set to 40 μ L.

V2.00+ : The Residual Volume is the volume that is not recovered after the sample is ejected from the syringe. This value is always 18 μ L \pm 2 μ L.

5.6.1.5 Port Number

The Port Number corresponds to the CPU serial port to which the HydroShear DNA Shearing Device is connected. Typically, the CPU port used is the COM1 port. The possible CPU ports used and the corresponding Port Number are listed underneath the “Port Number” heading and value text box (e.g. “0= COM1”). If an incorrect Port Number is entered, an “error 37” message will appear when the machine is used. (See 9.1.1 Message: Error 37 Occurred at Serial Port, page 67).

5.6.2 To Edit Machine Parameter Values

To set new parameter value default, follow these steps:

1. **Locate the text box next to each parameter name (e.g. “Volume”).**
2. **Either click in the text box and type in the desired value, or click the arrows next to the text box to display the desired value.**

5.6.3 To Save as New Default Values

1. **After entering values for each parameter, click the SAVE AS DEFAULT button.**
The new values are saved and are used as the default values for every shearing run.

5.6.4 To Restore Machine Parameter Default Values

If you have changed parameter values in the text boxes and wish to restore the default values:

1. **Click the RESTORE TO DEFAULTS button.**

The most recently saved default values are displayed in the text boxes. These values will be used as the default values for every shearing run.

5.6.5 To Restore Factory-Set Machine Parameter Default Values

If you have changed parameter values in the text boxes and/or saved parameter values as defaults and wish to restore the default values:

1. **Click the SET TO FACTORY DEFAULTS button.**

The default values determined for your machine by GeneMachines' engineers are displayed in the text boxes. These values will be used as the default values for every shearing run.

6. Shearing

This chapter offers step-by-step instructions on conducting a shearing run. Simply begin with 6.1 Set Shearing Parameters and continue through the chapter until 6.4 Shear the Sample. The last section, 6.5 Conducting the Wash Scheme, offers an in-depth explanation of and user instructions for the wash scheme.

Shearing Steps

6.1 Set Shearing Parameters

6.2 Filter Wash Solutions

6.3 Prepare the Sample

6.4 Shear the Sample

6.5 Conducting the Wash



WARNING: Wear safety goggles when operating the HydroShear DNA Shearing Device.

6.1 SET SHEARING PARAMETERS

Before conducting a shearing run, several parameters -- Volume, Number of Cycles (i.e. shearing passes), and Speed Code (i.e. pump speed) -- need to be specified on the HydroShear DNA Shearing Device software's Main Panel. (See 5.3 Shearing Parameters, page 37).

To enter a parameter value, follow these steps:

1. In the HydroShear DNA Shearing Device Main Panel's "Shearing Parameters" box, locate the text box next to a parameter name (e.g. "Volume").
2. Specify the desired parameter value. To do so, either click in the text box and type, or click on the arrows next to the text box.
3. Repeat Steps 1-2 to specify values for all three basic shearing parameters.

6.2 FILTER WASH SOLUTIONS

In order to prevent clogging of the shearing assembly, all wash solutions should be filter sterilized. GeneMachines recommends using a filter of no more than .2 microns.

GeneMachines does have a complete solution set available for purchase, utilizing 0.2 M HCl, 0.2 M NaOH, and 1 x TE buffer as Wash Solution I, II, and III, respectively. These solutions have passed stringent quality standards to ensure optimal results. (See *TABLE 1. HydroShear DNA Shearing Device Solution Set (Order #HSA-Kit-1)*, page 46).

TABLE 1. HydroShear DNA Shearing Device Solution Set (Order #HSA-Kit-1)

Wash Solution I	Wash Solution II	Wash Solution III
#HSA-WSI	#HSA-WSII	#HSA-WSIII
0.2 M HCl	0.2 M NaOH	1 x TE buffer, pH 8.0

6.3 PREPARE THE SAMPLE

Careful preparation of the sample is imperative! A clogged shearing assembly is the most common problem encountered by HydroShear DNA Shearing Device users, and the sample itself usually acts as the clogging material. The DNA must be thoroughly resuspended in solution before shearing.

- 1. Ensure that the DNA sample has been resuspended in distilled, deionized water (ddH₂O) or an appropriate buffer.**

GeneMachines recommends 1 x TE, pH 8.0.

- 2. Incubate the DNA in solution for 30 minutes at 37°C; vortex every 10 minutes during incubation.**
- 3. Immediately before shearing, centrifuge the sample at a speed of 14,000 rpm at room temperature for 15-25 minutes.**



- 4. If any pellet forms after centrifugation, then the DNA is not completely in solution. Follow these steps:**

- Incubate and vortex again. Then, repeat the centrifugation.
- Repeat the cycle until no pellet forms after centrifugation.

--You may need to dilute the sample with additional resuspension solution in order to achieve complete resuspension.

6.4 SHEAR THE SAMPLE

1. Click the **START** button located on the HydroShear DNA Shearing Device Main Panel.

A dialog box appears, displaying, “Do you want to wash the shearing device before starting the shearing process?”

2. Click **OK** unless the HydroShear DNA Shearing Device has just been cleaned. (This may be the case if you are conducting multiple shearing runs. Always clean the HydroShear DNA Shearing Device before the first run of the day, even if it was cleaned after the last run of the previous day).

The wash scheme specified is performed. For a step-by-step explanation of the on-screen prompts and corresponding user tasks, see 6.5 *Conducting the Wash Scheme*, page 49. Upon completion, a dialog box appears displaying, “Washing complete” and an **OK** button.

3. Click **OK**.

A dialog box appears, displaying, “Proceed with loading the sample?” and two buttons: **LOAD** and **BYPASS LOAD**. If the sample is already in the syringe, you should click **BYPASS LOAD**. In most cases, you will not have loaded the sample yet and should click **LOAD**.

4. Click **LOAD** (unless the sample is already in the syringe).

A dialog box appears, displaying, “Prepare to load the sample” and an **OK** button.

5. Make sure you have your sample prepared such that it is thoroughly in solution. (See 6.3 *Prepare the Sample*, page 46)

6. Click **OK**.

A dialog box appears displaying, “Turn valve to input, bring sample to input tube, and click **OK**.”

7. Turn the valve handle counterclockwise into the input position.

8. Hold the test tube containing the sample near the HydroShear DNA Shearing Device.

GeneMachines’ biologists recommend 1.5 mL as a convenient test tube size.

Please Note!

During the shearing procedure, you will need to perform steps to clear air bubbles. **This is normal.** However, the steps you will perform will be *contradictory* to the software prompts that appear. Follow these directions carefully and do adhere to them when they contradict software prompts.

FIGURE 6-1 Valve Handle in the Input Position

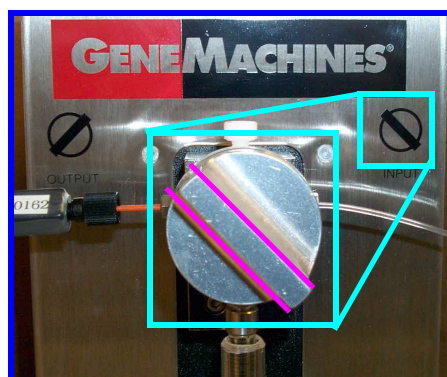


FIGURE 6-2 Valve Handle in the Output Position



-
9. **Place the HydroShear DNA Shearing Device's input tube into the sample tube, making sure it reaches the bottom.**
 10. **Click OK.**

The specified volume is drawn from the sample tube into the input tube.
A dialog box appears, displaying, "Remove sample from the input tube and click OK."
 11. **Take the input tube out of the sample tube. Discard or set aside the sample tube.**
 12. **Click OK.**

The sample is drawn into the syringe from the input tube.
A dialog box appears, displaying "Turn valve to output and click OK."
 13. **ONLY TURN THE VALVE HALFWAY, SUCH THAT THE VALVE HANDLE IS PERFECTLY VERTICAL (i.e. POINTING DOWNWARDS).**
 14. **Click OK and watch the bubble rise as air is pushed out of the syringe.**
 15. **Turn the output valve handle entirely into the output position, as soon as the bubble rises to the top of the sample.**



CAUTION: The pump will stall if the handle is not turned entirely to the output position

16. **A dialog box appears, displaying "Sample loading complete" and an OK button.**
17. **Click OK.**

A series of three messages will appear in a dialog box titled, "Clear Air Gap." Three buttons will also appear in the dialog box each time: **YES**, **NO**, and **ABORT PROTOCOL**. The first message asks, "Is there an air gap near the bottom of the syringe?"
18. **Click NO.**
19. **Click NO in response to the second and third Clear Air Gap messages.**

The Clear Air Gap dialog box disappears and a dialog box appears displaying, "Click OK to begin shearing cycles."
20. **Click OK.**

The machine conducts the number of shearing passes specified in the Shearing Parameters area of the HydroShear DNA Shearing Device Main Panel. The number of passes completed is constantly updated in the Status area of the HydroShear DNA Shearing Device Main Panel.
Upon completion of the shearing cycles, a dialog box appears displaying, "Shearing cycles complete" and an **OK** button.
21. **Click OK.**

A dialog box appears, displaying "Proceed to eject the sample from the device?" and a **YES** button.

22. Click YES.

A dialog box appears, displaying “Turn the valve to input and click OK.”

23. Click OK.

Air is drawn into the input tube, and a dialog box appears, displaying “Turn valve to output to eject sample” and an **OK** button.

24. Place the HydroShear DNA Shearing Device’s output tube into an appropriate collection vial.

25. Click OK.

The sheared sample is pushed through the HydroShear DNA Shearing Device’s output tube into the sample tube. A dialog box appears, displaying “Do you want to wash the device?” and a **YES** and **NO** button. GeneMachines recommends conducting the wash scheme after every shearing run and before the first shearing run of the day.

26. Click YES

The wash scheme chosen on the HydroShear DNA Shearing Device Main Panel and specified in the Wash Scheme window is completed. Clicking **NO** to skip the wash scheme is generally inadvisable.

6.5 CONDUCTING THE WASH SCHEME

After clicking the **START** button on the HydroShear DNA Shearing Device Main Panel and again after completing a shearing run, a dialog box appears displaying the message, “Do you want to wash the shearing device before starting the shearing process” and **YES** and **NO** buttons. Clicking **YES** will cause the machine to conduct its wash scheme. (See 5.3.3 *The Wash Scheme*, page 38). The following describes the wash scheme and the steps you will be asked to carry out during the washing process:

- 1. A dialog box appears displaying the message, “Do you want to wash the shearing device before starting the shearing process” and YES and NO buttons.**
- 2. Place the output tube into a waste container.**

GeneMachines offers a convenient waste bottle for use with the HydroShear DNA Shearing Device. To order, contact GeneMachines. (See 1.4 *Contacting GeneMachines®*, page 10)
- 3. Click YES.**

A dialog box appears displaying, “Turn valve to input and click OK.”
- 4. Turn the valve handle counterclockwise into the input position.**
- 5. Click OK.**

A dialog box appears displaying, “Bring <name of Wash Solution I> to input tube and click OK.” The actual wash solution name that appears is the user-chosen name entered in the Wash Scheme Window. (See 5.3.3 *The Wash Scheme*, page 38).
- 6. Hold the bottle containing Wash Solution I (as specified in the Wash Scheme Window) near the HydroShear DNA Shearing Device. (See 5.3.3 *The Wash Scheme*, page 38)**

-
- 7. Place the HydroShear DNA Shearing Device's input tube into the Wash Solution I bottle.**

 - 8. Click OK.**

The syringe plunger lowers, and Wash Solution I is drawn into the pump.
A dialog box appears displaying, "Turn valve to output and click OK."

 - 9. Turn the valve handle clockwise into the output position.**

 - 10. Click OK.**

The syringe plunger rises into the syringe, and Wash Solution I is pushed out of the pump, through the output tube, and into the waste container.

 - 11. As the screen prompts repeat, repeat steps 4-10 accordingly until the number of Wash Solution I cycles specified in the wash scheme have been completed.**

The messages will then repeat again, asking you to use <name of Wash Solution II> and <name of Wash Solution III>. The Wash Solution names used are the user-chosen names entered in the Wash Scheme Window. (*See 5.3.3 The Wash Scheme, page 38*).

 - 12. Repeat steps 4-10, using Wash Solution II and then Wash Solution III (as prompted) until all wash cycles have been completed.**

Washing is now complete, but two more pump passes must be executed in order to flush leftover washing solution from the machine. The steps below describe the steps involved in completing these passes.

 - 13. A dialog box appears, displaying "Remove any liquids from the opening of the input tube and click OK." Remove the input tube from the Wash Solution III bottle and allow it to hang free in the open air.**

 - 14. Click OK.**

A dialog box appears displaying, "Turn valve to input and click OK."

 - 15. Turn the valve handle into the input position.**

 - 16. Click OK.**

The syringe plunger lowers, causing air to be drawn into the input tube. This air pushes any washing solution that has remained in the input tube into the syringe.
A dialog box appears displaying, "Turn valve to output and click OK."

 - 17. Turn the valve handle clockwise into the output position.**

 - 18. Click OK.**

The syringe plunger rises into the syringe, and the leftover wash solution is pushed from the syringe into the output tube.

 - 19. A dialog box appears displaying, "Turn valve to input and click OK."**

 - 20. Turn the valve handle into the input position.**

21. Click OK.

The syringe plunger lowers, causing more air to be drawn into the syringe.
A dialog box appears displaying, “Turn valve to output and click OK.”

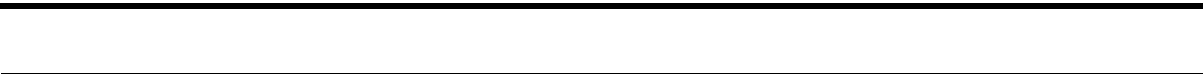
22. Turn the valve handle clockwise into the output position.

23. Click OK.

The syringe plunger rises into the syringe, pushing air into the output tube. This air pushes the leftover wash solution out of the output tube and into the waste container.
A dialog box appears, displaying, “Washing Complete?”

24. Click OK.

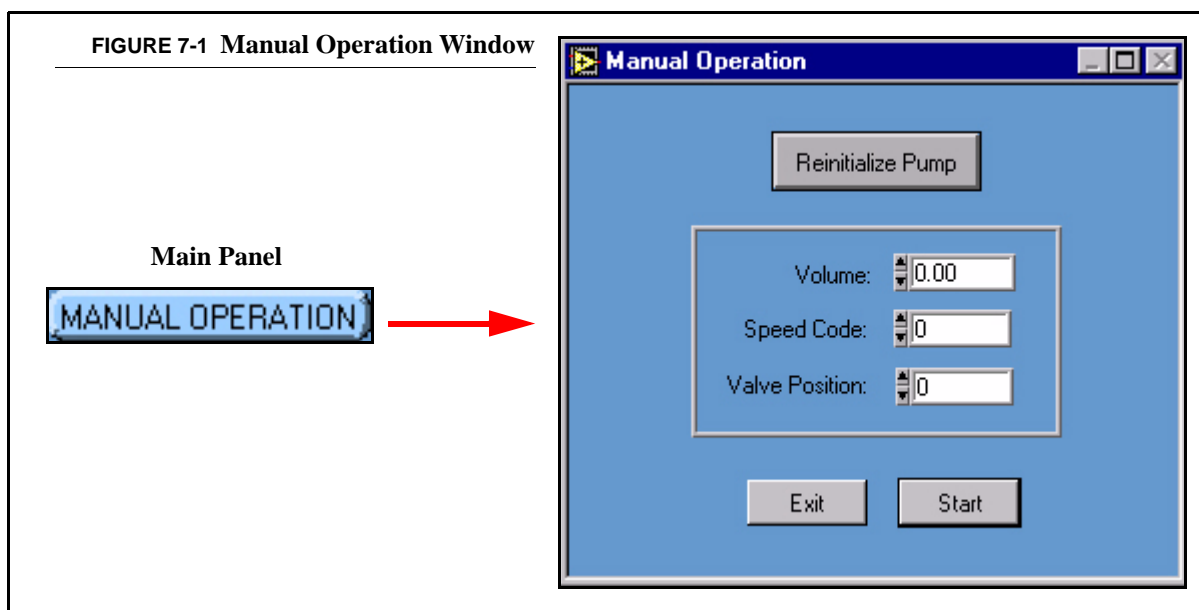
The HydroShear DNA Shearing Device is now clean and ready to conduct a shearing run or be left overnight.



7. Manual Operation

Individual shearing steps can be manually controlled using the Manual Operation window. Three functions are easily carried out from the Manual Operation window: bringing fluid into the syringe, ejecting fluid from the syringe, and reinitializing the pump.

To reach the Manual Operation window, click the Manual Operation button on the **HYDROSHEAR DNA SHEARING DEVICE MAIN PANEL**.



7.1 MANUAL OPERATION: THE BASICS

- Each time the **START** button is clicked, the pump will complete one pass of the indicated volume and speed.
- Pursuant to the sign (+/-) of the Volume value specified, fluid or air is either drawn into the syringe or expelled from the syringe. A positive (+) sign causes fluid or air to be drawn into the syringe; a negative (-) sign causes fluid or air to be pushed out.
- GeneMachines recommends reinitializing the pump after manual operation.

7.2 DRAWING VOLUME INTO THE PUMP

The following procedure should only be used when there is no volume in the syringe. To draw volume (fluid or air) into the pump, follow these steps:

1. **Locate the Status box on right-hand side of the HydroShear DNA Shearing Device Main Window.**
2. **Verify that “0.00” appears in the text box headed “Current pump volume” to indicate that there is no fluid in the pump.**
3. **Turn the valve handle counterclockwise to the input position.**
(See FIGURE 2-1 Valve Knob Orientations, page 14).
4. **In the text box headed “Volume,” specify a *positive* Volume value. To do so, either click in the text box and type or click on the arrows next to the text box.**
In order to draw volume into the pump, the Volume value must be positive.
5. **In the text box headed “Speed Code,” specify a pump speed. (See 5.3.1.3 Speed Code, page 38). To do so, either click in the text box and type or click on the arrows next to the text box.**
For manual operation, 10 is a reasonable Speed Code. Choose 20 if the shearing assembly is clogged or if you want a slow syringe movement.
6. **Click the START button.**
One pump pass is conducted at the specified Speed Code and the specified volume is drawn into the syringe.
7. **If you are finished manually operating the pump, click the REINITIALIZE PUMP button to reinitialize the pump.**
8. **Click the EXIT button.**
The Manual Operation window disappears. The HydroShear DNA Shearing Device Main Panel appears.

7.3 EJECTING FLUID FROM THE PUMP

The following procedure should only be used when there is no volume in the syringe. To draw volume into the pump, follow these steps:

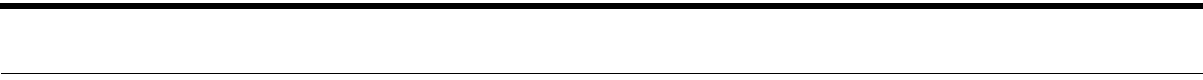
1. **Locate the Status box on right-hand side of the HydroShear DNA Shearing Device Main Window.**
2. **Look in the text box headed “Current pump volume” to determine the current pump volume.**
3. **Click the MANUAL OPERATION button on the Main Panel.**
The Manual Operation window appears.

4. **In the text box headed “Volume,” specify the *negative* value of the current pump volume determined in Step 2. To do so, either click in the text box and type or click on the arrows next to the text box.**
For example, if the Status box displays that the current pump volume is 400, enter -400 in the “Volume” text box.
5. **In the text box headed “Speed Code,” specify a pump speed. (See 5.3.1.3 *Speed Code*, page 38). To do so, either click in the text box and type or click on the arrows next to the text box.**
For manual operation, 10 is a reasonable speed code. Choose 20 if the syringe is clogged or if you want a slow syringe movement.
6. **Turn the valve handle clockwise to the output position.**
(See FIGURE 2-1 *Valve Knob Orientations*, page 14).
7. **Place the open end of the output tube inside an empty test tube or other collection vial in preparation for sample collection.**
8. **Click the START button.**
The sample is ejected into the tube.
9. **If you are finished manually operating the pump, click the REINITIALIZE PUMP button to reinitialize the pump.**
10. **Click the EXIT button.**
The Manual Operation window disappears. The HydroShear DNA Shearing Device Main Panel appears.

7.4 REINITIALIZING THE PUMP

To reinitialize the pump:

1. **Click the REINITIALIZE PUMP button.**
2. **Click the EXIT button.**
The Manual Operation window disappears. The HydroShear DNA Shearing Device Main Panel appears.



8. Maintenance

8.1 ROUTINE

8.1.1 Daily Maintenance Guidelines

- Keep the HydroShear DNA Shearing Device covered when not in use; dust can reduce the life of the valve.
- Flush the pump out thoroughly after each use. Use distilled or deionized water.
- The pump should be primed with distilled or deionized water when not in use.
- The pump should not be run dry for more than a few cycles.
- Inspect the pump for leaks; correct any problems immediately.
- Immediately wipe up spills on and around the HydroShear DNA Shearing Device.

8.1.2 Periodic Cleaning

The cleaning regimens below are included as options for users who feel that their machine requires extensive cleaning or who have neglected the daily maintenance tasks. **However, if you adhere to the guidelines in 8.1.1 Daily Maintenance Guidelines, page 57, the extensive cleaning regimens detailed in this section should not be necessary.**

Definitions of Cleaning Tasks:

Prime -- To draw fluid into. (See 7.2 Drawing Volume into the Pump, page 54).

Flush -- To draw fluid into and then expel it. (See 7.2 Drawing Volume into the Pump, page 54, and 7.3 Ejecting Fluid from the Pump, page 54).

Cycle into waste -- To eject fluid into a waste container. (See 7.3 Ejecting Fluid from the Pump, page 54)

The HydroShear DNA Shearing Device's fluid path should be thoroughly cleaned using any one of the three procedures outlined below:

8.1.2.1 Cleaning with Weak Detergent

1. Prime the pump with a weak detergent solution.
2. Allow the detergent to remain in the pump, with the syringe fully lowered, for 30 minutes.

-
3. After the 30-minute period, remove the reagent tubing from the detergent.
 4. Cycle the fluid from the syringes and tubing into a waste container.
 5. Prime the pump for at least 10 cycles with distilled or deionized water.
 6. Allow the water to remain in the fluid pathways (tubings, orifice box, etc.) for storage.

8.1.2.2 Cleaning with Weak Acid and Base in Sequence

1. Prime the pump with 0.2M NaOH.
2. Allow the 0.2M NaOH to remain in the pump, with the syringe fully lowered, for 10 minutes.
3. After the 10-minute period, remove the reagent tubing from the 0.2M NaOH.
4. Flush the pump with distilled or deionized water.
5. Prime the pump with the 0.2M HCl.
6. Allow the 0.2M HCl to remain in the pump, with the syringe fully lowered, for 10 minutes.
7. After the 10-minute period, remove the reagent tubing from the 0.2M HCl.
8. Cycle all the fluid from the syringe and tubing into a waste container.
9. Prime the pump for at least 10 cycles with distilled or deionized water.
10. Allow the water to remain in the fluid pathways (tubings, orifice box, etc.) for storage.

8.1.2.3 Cleaning with 10% Bleach

1. Prepare a 10% bleach solution by adding 1 part commercial bleach to 9 parts water.
2. Prime the pump with the 10% bleach solution.
3. Allow the solution to remain in the pump, with the syringe fully lowered, for 30 minutes.
4. After the 30-minute period, remove the reagent tubing from the 10% bleach solution.
5. Cycle all the fluid from the syringe and tubing into a waste container.
6. Prime the pump for at least 10 cycles with distilled or deionized water.
7. Allow the water to remain in the fluid pathways (tubings, orifice box, etc.) for storage.

8.2 PERIODIC MAINTENANCE



Visually check the alignment of the syringe after conducting any maintenance procedures. If the syringe does not appear to be parallel to the HydroShear DNA Shearing Device case, some component is misaligned. Check the position of the valve within the valve mount; also check the orientation of the valve mount.

8.2.1 Unclogging Shearing Orifices -- Sonication Protocol

Sonication will unclog shearing orifices. Any sonication device can be used. If you are purchasing a sonication device specifically for this purpose, GeneMachines recommends the Fisher Ultrasonic Cleaner, Model FS6, 18 oz. capacity.

Depending on the severity of the clog, it may not be necessary to complete all the steps in the sonication/unclogging procedure below. However, always be sure to end with a water wash. (See *Wash 4: Water Wash*, page 61).

To unclog (a) shearing orifice(s) by sonication, follow these steps:

Preparation

- 1. Disconnect the shearing assembly from the main tower.**
- 2. Unscrew the nuts connecting all tubing (the output tube and the tubing leading to the base unit screw) to the orifice box.**
(See *FIGURE 4-3 A Shearing Assembly*, page 30).
- 3. Screw a 1/16" screw into the base screw's port on the shearing tower.**
This screw will prevent dust from entering the shearing tower while the shearing assembly is detached.

Wash 1: Detergent and Water Wash

- 4. Prepare a solution of detergent and water.**
- 5. Pour the detergent and water solution into a beaker.**
Add enough solution so that the shearing orifice box(es) will be completely covered when they are placed in the beaker.
- 6. Place the shearing orifice box(es) into the beaker.**
- 7. Fill the sonicator with enough water so that the sonicator's water line will be higher than the beaker's water line when the beaker is placed in the sonicator.**

-
8. Place the beaker, with the shearing orifice box(es) inside, into the sonicator.
 9. Look through the beaker at the shearing devices to make sure that no air bubbles are blocking the center of the orifice box(es).
If air bubbles are present:
While submerging the orifice box in water, use a pipet tip to either dislodge the bubble or pipet out the bubble. Take care not to contact the ruby in the center of an orifice box with the pipet tip; the calibration of the orifice may be compromised if you do.
 10. Sonicate for 10-15 minutes.
 11. After sonicating, pour the detergent and water solution out of the beaker.
 12. Rinse the orifice box(es) with water by leaving it (them) in the beaker and rinsing the entire beaker with water.

Wash 2: Water Wash

13. Add water to the beaker, with orifice box(es) still inside.
14. Look through the beaker at the shearing devices to make sure that no air bubbles are blocking the center of the orifice box(es).
If air bubbles are present:
While submerging the orifice box(es) in water, use a pipet tip to remove the air bubbles. Take care not to contact the center of an orifice box with the pipet tip; the calibration of the orifice may be compromised if you do.
15. Sonicate for 10-15 minutes.
16. Pour the water out of the beaker, leaving the orifice boxes inside.

Wash 3: Alcohol Wash

17. Pour isopropyl alcohol into the beaker until the orifice boxes are submerged.
18. Look through the beaker at the shearing devices to make sure that no air bubbles are blocking the center of the orifice box(es).
If air bubbles are present:
While submerging the orifice box(es) in water, use a pipet tip to remove any air bubbles. Take care not to contact the center of an orifice box with the pipet tip; if you do, the calibration of the orifice box may be compromised.
19. Place the beaker into the sonicator.
20. Sonicate for 10-15 minutes.
21. Pour the alcohol out of the beaker.
22. Rinse the orifice box(es) with water by leaving them in the beaker and rinsing the entire beaker with water.

Wash 4: Water Wash

23. Add water to the beaker with the orifice box(es) still inside.
24. Place the beaker into the sonicator.
25. Sonicate for 10-15 minutes.
26. Pour the water out of the beaker.
27. Let the orifice box(es) dry for at least 30 minutes.

8.2.2 Replacing Tubing

In order to ensure proper shearing, tubing must be kept clean and free from crimps. Dirty, blocked, or crimped tubing can result in poor accuracy, loss of precision, loss of the appropriate air gap, and syringe stalling. Replacement tubing can be ordered from GeneMachines. (See 1.4 Contacting GeneMachines®, page 10).

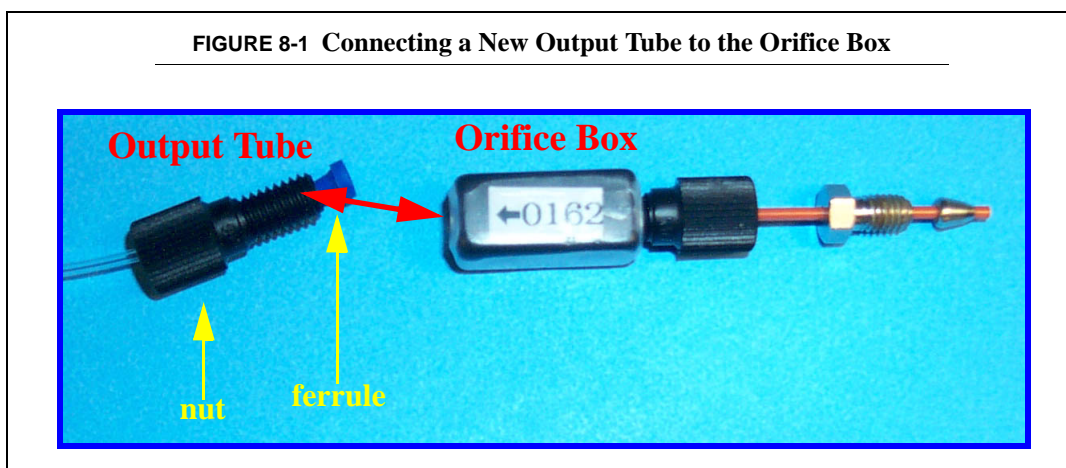
8.2.2.1 Replacing the Output Tube

To replace output tubing, follow these steps:

Each new shearing assembly has a new output tube pre-attached. If necessary, you can replace the output tube without replacing the entire shearing assembly.

Each piece of replacement output tubing has a ferrule and nut attached. The tapered end of the ferrule should be closer to the nut. The threaded end of the nut should face the ferrule. (FIGURE 8-1)

1. **Insert the ferrule into the shearing orifice box's output tubing port.**
This port is located at the point of the arrow label on the shearing orifice box. (FIGURE 8-1)
2. **Screw the nut into the port.**



8.2.2.2 Replacing the Input Tube

To replace input tubing, follow these steps:

Each piece of input tubing has an attached metal ferrule.

1. Using the 1/4" hex wrench provided in the HydroShear DNA Shearing Device tool kit, loosen the hex screw that attaches the input tube to the valve.
2. Use your fingers to finish unscrewing the hex screw from the valve.
3. Pull the old tubing out of the hex screw.
4. Insert the free end (i.e. the end without a ferrule) of the tubing into the threaded end of the hex screw.
When surrounding the tubing, the threaded end of the hex screw should face the tapered end of the metal ferrule.
5. Insert the ferrule of the new input tube into the port on the right side of the valve.
6. Screw the hex screw into the port.
7. Use the 1/4" hex wrench to secure the hex screw into the port.

FIGURE 8-2 Replacing the Input Tube

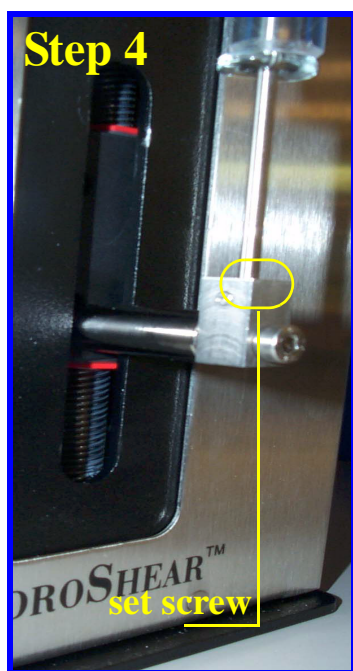


8.2.3 Syringe Maintenance

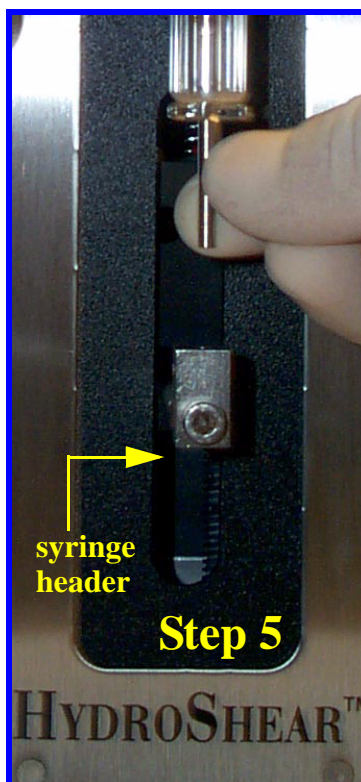
8.2.3.1 Removing the Syringe

1. Using the Manual Operation Window, draw approximately 250 μL of air into the syringe.
(See 7.2 Drawing Volume into the Pump, page 54).
2. Turn the pump off, using the power switch located at the bottom right of the machine.
3. Disconnect the power cable from the wall outlet.
4. Using the 0.050" Allen wrench provided in the HydroShear DNA Shearing Device tool kit, loosen the small plunger set screw, located on the left side of the syringe mount.
5. Carefully push the plunger upwards until it is completely free from the syringe mount.
6. Gently grasp the syringe and twist counterclockwise to unscrew the syringe from the syringe adapter.

FIGURE 8-3 Removing the Syringe



The set screw on the syringe mount.



Pushing the plunger up into the syringe mount.



Unscrewing the syringe from the syringe adapter.

8.2.3.2 Replacing the Syringe Adapter

The syringe adapter will periodically need to be replaced. A worn syringe adapter may cause the following problems:

- Poor precision and accuracy
- Variability or movement of the air gap
- Fluid leaks from the top of the syringe
- Problems bringing fluid into the syringe
- Puncturing of the syringe seal by the plunger

--If the plunger punctures the syringe seal, it may scratch the inside of the glass syringe. If scratching does occur, the entire syringe needs to be replaced.

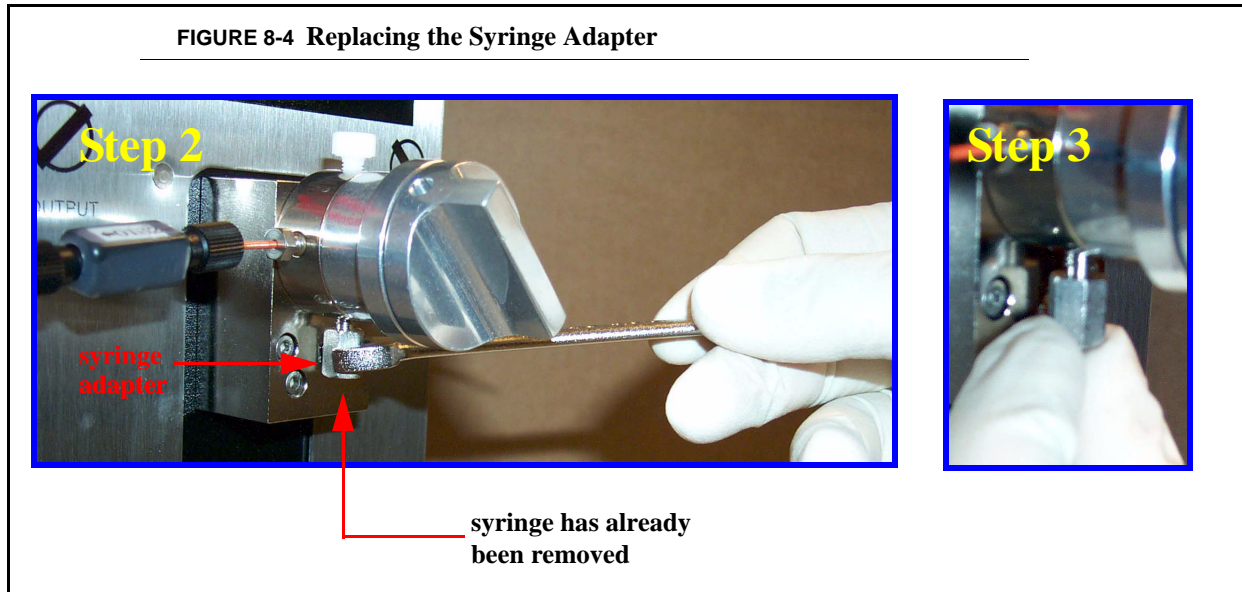
The frequency of replacement depends on several factors, including

- Frequency of use
- Types of fluids being used in the machine
- Size of the syringe
- How well the machine is maintained

To replace the syringe adapter, follow these steps:

- 1. Remove the syringe from the HydroShear DNA Shearing Device.**
(See 8.2.3.1 Removing the Syringe, page 63)
- 2. Using the 5/16" hex wrench provided in the HydroShear DNA Shearing Device tool kit, loosen the syringe adapter. (FIGURE 8-4)**
- 3. Use your fingers to finish unscrewing the syringe adapter.**
- 4. Screw the new syringe adapter in place and tighten with the 5/16" hex wrench.**
- 5. Reattach the syringe.**
(See 4.2 Syringe Installation, page 27)

FIGURE 8-4 Replacing the Syringe Adapter



8.2.4 Lead Screw Maintenance

8.2.4.1 Lubricating the Lead Screw

If the pump is making a “screeching” noise or if the syringe is stalling frequently, the lead screw needs to be lubricated. Lubrication is usually only needed after 700,000 syringe strokes. If the lead screw seems to require more frequent lubrication, suspect another problem.

To lubricate the lead screw, follow these steps:

1. Use a skewer, toothpick, or cotton swab to spread a generous layer of Cavro lubricant along the lead screw.
2. Conduct a shearing run, or, use the Manual Operation Window to retract and expel fluid or air from the syringe several times.
The Cavro lubricant will be spread along the lead screw during operation of the syringe.

8.2.5 Valve Maintenance

8.2.5.1 Replacing the Valve Assembly

To replace the valve assembly, follow these steps:

1. Using the Manual Operation Window, draw approximately 250 μL of air into the syringe.
(See 7.2 Drawing Volume into the Pump, page 54).
 2. Turn the pump off, using the power switch located at the bottom right of the machine.
-

-
3. **Disconnect the power cable from the wall.**
 4. **Detach the shearing assembly from the valve by unscrewing the base screw. Set aside in a dust-free location.**
 5. **Detach the input tube from the valve by unscrewing the nut. Set aside in a dust-free location.**
 6. **Remove the syringe from the machine.**
(See 8.2.3.1 Removing the Syringe, page 63).
 7. **Using the 0.050" Allen wrench included in the HydroShear DNA Shearing Device tool kit, unscrew the valve set screw located along the top edge of the valve handle.**
 8. **Carefully remove the handle from the machine and set aside.**
 9. **Using a 2.5 mm hex wrench, unscrew and remove the two screws on the face of the valve assembly. These two screws secure the valve assembly to the pump.**
One of the screws is located at the top right of the valve assembly face; the other is located at the bottom left of the valve assembly face.
 10. **Carefully pull the entire valve assembly out of the valve mount.**

9. Troubleshooting

9.1 ERROR MESSAGES

9.1.1 Message: Error 37 Occurred at Serial Port

This message will appear if the wrong CPU serial port number is entered as the Port Number in the Machine Parameters window.

- Check to see that the correct CPU serial port number is entered in the Port Number text box on the Machine Parameters window.

9.1.2 Reading Time Out

This error message appears when the HydroShear DNA Shearing Device and control computer are not communicating.

To resolve this error, follow these steps:

1. **Make sure the HydroShear DNA Shearing Device is plugged in and turned on.**
2. **Check to see that the correct CPU serial port number is entered in the Port Number text box on the Machine Parameters window.**
3. **Check to make sure that the serial cable is securely connected to both the HydroShear DNA Shearing Device's serial port and the CPU's serial port.**

9.1.3 Pump Overload or Plunger Overload

This error message appears when the pressure within the syringe becomes so high that the syringe could break. This situation is most commonly caused by a clogged shearing assembly.

- Reinitialize the pump.
If the error message again appears, the shearing assembly is probably clogged. Unclog the shearing assembly. (See 8.2.1 *Unclogging Shearing Orifices -- Sonication Protocol*, page 59).

9.1.4 Other Error Messages

Miscellaneous error messages will appear if the pump receives commands from the software that are illogical.

- To resolve the error, initialize the pump.

9.2 PROBLEMS VISIBLE DURING SHEARING

9.2.1 I see a bubble inside the syringe.

This is normal. During the shearing procedure, you will need to perform steps to clear air bubbles. However, the steps you will perform will be *contradictory* to the software prompts that appear. Carefully follow the directions in [6.4 Shear the Sample, page 47](#), and do adhere to them when they contradict software prompts.

9.2.2 I see an air bubble in the syringe when the sample is retracted from the output tube.

- The shearing assembly may be partially clogged.
 1. Click the **STOP** button to stop the shearing run. (*See 5.4 Operation Controls, page 40*).
 2. Use the Manual Operation Window to eject the DNA sample. (*See 7.3 Ejecting Fluid from the Pump, page 54*). Save the DNA sample for when you resume the shearing run.
 3. Unclog the shearing assembly. (*See 8.2.1 Unclogging Shearing Orifices -- Sonication Protocol, page 59*).

9.2.3 I see frothy bubbles in the sample when it leaves the orifice.

- The shearing assembly may be partially clogged.
 1. Click the **STOP** button to stop the shearing run. (*See 5.4 Operation Controls, page 40*).
 2. Use the Manual Operation Window to eject the DNA sample. (*See 7.3 Ejecting Fluid from the Pump, page 54*). Save the DNA sample for when you resume the shearing run.
 3. Unclog the shearing assembly. (*See 8.2.1 Unclogging Shearing Orifices -- Sonication Protocol, page 59*).

9.2.4 The sample is retracted back into the syringe (from the output tube) at a sporadic rate.

- The shearing assembly may be partially clogged.
 1. Click the **STOP** button to stop the shearing run. (*See 5.4 Operation Controls, page 40*).
 2. Use the Manual Operation Window to eject the DNA sample. (*See 7.3 Ejecting Fluid from the Pump, page 54*). Save the DNA sample for when you resume the shearing run.
 3. Unclog the shearing assembly. (*See 8.2.1 Unclogging Shearing Orifices -- Sonication Protocol, page 59*).

9.2.5 The sample continues to move after the syringe has finished retracting.

- The shearing assembly may be partially clogged.
 1. Click the **STOP** button to stop the shearing run. (See 5.4 Operation Controls, page 40).
 2. Use the Manual Operation Window to eject the DNA sample. (See 7.3 Ejecting Fluid from the Pump, page 54). Save the DNA sample for when you resume the shearing run.
 3. Unclog the shearing assembly. (See 8.2.1 Unclogging Shearing Orifices -- Sonication Protocol, page 59).

9.2.6 The volume of sample seems to be decreasing.

- Most likely, a fitting, valve, or the syringe is leaking. Tighten all the tubing connections and screws. If the problem persists, Contact GeneMachines for assistance. (See 1.4 Contacting GeneMachines®, page 10).

9.3 SAMPLE AND FRAGMENT PROBLEMS

9.3.1 DNA is lost

Losing DNA is most likely a result of inadequate purging of the syringe after washing. If the syringe is not properly purged after washing, leftover wash solution may damage or destroy DNA.

9.3.2 The fragments produced aren't the right size

There are three likely causes of incorrect fragment size.

1. **Air bubbles were in the sample during calibration or sample shearing.**

During the next shearing run, carefully follow the shearing procedure in 6.4 Shear the Sample, page 47. This procedure includes the steps necessary to remove air bubbles from the sample. If you successfully remove air bubbles and complete the run and the fragments produced are again the wrong size, proceed to the next possible cause of incorrect fragment size, 2. *The shearing orifice is partially clogged., page 69.*
2. **The shearing orifice is partially clogged.**
 1. Click the **STOP** button to stop the shearing run. (See 5.4 Operation Controls, page 40).
 2. Use the Manual Operation Window to eject the DNA sample. (See 7.3 Ejecting Fluid from the Pump, page 54). Save the DNA sample for when you resume the shearing run.
 3. Unclog the shearing assembly. (See 8.2.1 Unclogging Shearing Orifices -- Sonication Protocol, page 59).

If you follow the unclogging procedure and the fragments produced are still the wrong size, recalibrate the shearing assembly. (See 4.5.4 Calibrating the Shearing Assembly, page 33). If the fragments produced are not

consistently the correct size after using the new Speed Code:size values, proceed to the next possible cause of incorrect fragment size, *3. The shearing assembly may have been dropped or mishandled and damaged.*, page 70.

3. The shearing assembly may have been dropped or mishandled and damaged.

Discard the shearing assembly. Calibrate and then use a new shearing assembly.

9.3.3 The fragments produced do not clone well.

Poor cloning can be caused by many factors unrelated to the shearing process or to the sheared DNA. However, solutions used in the shearing process could damage or destroy DNA. Therefore, if the syringe was inadequately purged after washing, leftover wash solution could have damaged or destroyed the DNA being sheared.

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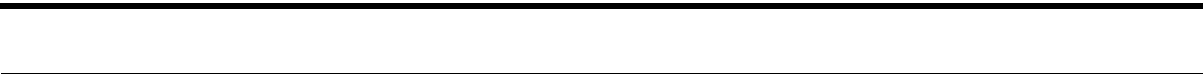
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Decontamination of Hazardous Chemicals, Radioactive and Biohazards Materials

Please be aware that the California Code of Regulations, Title 8 §5193(g), which addresses servicing or shipping contaminated equipment, states:

“Equipment which may become contaminated with blood or OPIM (other potentially infectious materials) shall be examined prior to servicing or shipping and shall be decontaminated as necessary, unless the employer can demonstrate that decontamination of such equipment or portions of such equipment is not feasible or will interfere with a manufacturer's ability to evaluate failure of the device.

1. A readily observable label, in accordance with subsection (g)(1)(A), shall be attached to the equipment stating which portions remain contaminated.

2. Information concerning all remaining contamination shall be conveyed to all affected employees, the servicing representative, and/or the manufacturer, as appropriate, prior to handling, servicing, or shipping so that appropriate precautions will be taken.”

All products returned to GeneMachines shall comply with California Code referenced above or Federal Code with regard to blood and OPIM, plus radioactive materials and hazardous chemicals. In addition, Customers must ship goods in compliance with commercial carrier standards and Department of Transportation (DOT) Regulations.

1. If your lab works with radioactive materials, please confirm that no radioactivity is detected on the machine using appropriate radiological detection methods and procedures. This is necessary even if radioactive material was not specifically used in the machine.
2. Please clean all exposed areas with 70% EtOH, bleach or other appropriate decontamination agent(s) for the biohazardous material(s) of concern.
3. Please report all remaining contaminants in the “Notes” section specified below if despite your best efforts to remove all radioactive and infectious biohazards, some contaminants remain.

Equipment Description:

Product Name:

Serial No.:

Order No.:

Quantity:

Place ‘X’ in Box A if applicable. Otherwise complete all parts of B, providing further information as requested or appropriate.

A. This equipment has not been in contact with unfixed biological agents, radioactive materials, or hazardous chemicals.

B. 1. This equipment has been exposed internally or externally to hazardous materials as indicated below:

Yes/No Blood, body fluids, pathological agents
 Yes/No Other biohazardous materials
 Yes/No Chemicals hazardous to health
 Yes/No Radioactive materials
 Yes/No Other hazardous materials

Please provide further details/notes here:

2. This equipment has been cleaned and decontaminated:

If Yes, please give details/notes of methods:

If No, please indicate why not:

3. The equipment has been prepared to ensure safe handling/transportation. Yes/No

Please sign and date this document confirming that the equipment that you are shipping to GeneMachines is free of all hazardous contaminants. We will not service any product without this signed form. Should GeneMachines consider a returned good a hazard, it will be returned immediately to the customer at his/her expense. Moreover, if hazardous contaminants are detected on a product received without a prior written notice of the contamination, GeneMachines will report customer to the appropriate authorities and charge direct cost of \$4,000 or more to the customer. In addition, GeneMachines will pursue all damages incurred resulting from customer's failure to abide by CCR Title 8 and other health and safety requirements imposed by law.

Signature: Date: Name: Title: Email: Telephone Number: Ext. Facsimile Number: Institute/Company: Department: Address:

Please do not write below this line. It is for GeneMachines internal use only.



Comments:

Employee Signature: Printed Name: Date:

Passed Inspection: Yes No