

## HydroShear Support (Q & A)

*What software version should I use?*

Digilab recommends using software version 2.0.9 with the regular HydroShear, and version 2.1.1 with the HydroShear Plus system. Please check the web page ([www.digilabglobal.com](http://www.digilabglobal.com)) periodically to see whether newer software versions compatible with your system have been released.

*How often should I wash the system?*

Digilab recommends performing sufficient wash steps in between DNA shearing procedures because it greatly reduces the risk of both clogging and cross-contamination. Theoretically, each washing step reduces the amount of residual DNA in the system by ten (so our standard wash procedure should reduce the risk of cross-contaminating your next sample  $10^{12}$ -fold). Sometimes, when there is repeated shearing of the same sample, cross-contamination may not be a problem, unless your application asks for the absence of non-sheared DNA in the sample. In the latter case, using the washing procedure after completing each shearing experiment is strongly recommended. Digilab recommends using our wash solution kit where each solution is certified to be particle and nuclease free and totally compatible with both HydroShear and HydroShearPlus systems.

*There is a bubble in the syringe*

There are instructions in the manual on how to get bubble(s) out of the syringe. It may take several times of repeating of the procedure to complete the task. If you are still unable to get the bubble(s) out, you should check the input tubing to make sure the input tubing is screwed in tightly. If it is tight, make sure it is not worn out as it may need replacing.

*I got “pump overloaded” error message*

This may be because the shearing speed selected is too fast. The standard shearing assembly usually goes down to speed code 3 or 4 but the small shearing assembly usually only works down to speed code 6. Because each shearing assembly is unique, each new shearing assembly should be calibrated before its initial use. This is to determine the fastest speed that the shearing assembly can handle. Pass pre-filtered water/buffer through the system at different speed codes until you reach the “pump overload” error message. If the “pump overload” message is received while using speed code 3, then 4 is the lowest speed code (fastest speed) to safely use with this shearing assembly.

If an appropriate speed code number is being used and the error message is still received, than the most probable reason for this is clogging of the shearing assembly. Clogging is not an age-dependent issue, although older and more frequently used shearing assemblies are more prone to become clogged.

*My shearing assembly is clogged.*

The best way to determine whether a shearing assembly is indeed clogged is to inspect the black box (orifice) under a microscope. To do so, disconnect the black box from the connectors and take a look inside the box using a 10x by 10x microscope (inverse microscope is more convenient to use). Normally, a white circle (hole) in the pink background (ruby) can easily be viewed. If there appears to be something blocking the hole (piece of dust, most often), it means the shearing assembly got clogged. If this is the case, try to unclog the unit by treating the black box in an ultrasound bath for 10-15 min in a beaker with distilled water (refer to Digilab manual for more detailed instructions: Chapter 8.2.1 for regular HydroShear; Chapter 7.4 for HydroShear Plus unit).

After the box has been treated, check it under the microscope again to confirm unclogging. Re-assemble the unit as specified in the manual (Chapter 4.5 for Regular HydroShear; Chapter 3.6 for the HydroShear Plus model); it is important that both connectors are screwed in to the orifice box simultaneously and that you do not over-tighten them. Check the newly assembled unit with water in manual mode to find out whether it works fine (look for any leakage problems).

*Shearing assembly keeps clogging*

Unfortunately, this may happen quite often unless you follow two simple rules.

1. Always use pre-filtered wash solutions (Digilab certified wash solutions are recommended). Keep such solutions in provided bottles (carefully wash off the bottles before use to remove any possible contaminations occurring during the shipment), tightly closed and connected to the system after opening/preparation of the solution. Digilab wash solutions are guaranteed to be particle-free until opened. After these bottles are opened, only the user can control the quality of the solutions. Several tasks are recommended to reduce the risk of contamination of the wash solutions:
  - a) Additional caps with pierced holes for the tubing are provided. Prior to changing the original caps for the pierced caps, wash the pierced caps with pre-filtered water/buffer to remove dust or particles possibly accumulated on the pierced cap;
  - b) Be sure to use the pierced caps to reduce or eliminate the chance of dust being deposited into the bottles;
  - c) Wash and clean the tubing before inserting it into the bottle with wash solution;
2. The second rule is to make sure the starting DNA sample contains no particulates. The best way to do so is through pre-filtering of the sample before injecting it into the HydroShear. If filter sterilization on the DNA sample is not desirable, at least centrifuge the sample at max speed (12,000-15,000 RPM) for 10-15 min and take the upper portion of the solution avoiding disturbing the pellet at the bottom of the tube.

Following these rules is even more essential when working with a small shearing assembly because it has a smaller hole in the orifice which can get blocked easily. Operator should start out with a speed code of 12 and work their way down to 6. DNA sample needs to be thoroughly re-suspended in solution and filtered before shearing. The user needs to take extra precaution to make sure no dust particles get into the sample before it reaches orifice.

Below are a couple of names of the filters which customers can use for DNA filtration. In general, any filter which is specified as a low-DNA binding one, or is recommended for DNA filtration, can be used. The major concern in choosing the filter is that the size of the filter pores should be significantly smaller than the size of the hole in the shearing assembly (which is about 60  $\mu\text{m}$  for standard assembly and about 40  $\mu\text{m}$  for small assembly). So, using a filter with pore diameter less than 10  $\mu\text{m}$  would be advisable (the smaller the pore size, the better, but filters with too small of a pore size might be difficult to use with DNA). Digilab recommends using glass fiber filters instead of nitrocellulose because of higher non-specific adsorption of DNA to nitrocellulose. (Also, it is possible to re-use the glass fiber filters after cleaning them in strong acid; the glass filters can be autoclaved and thus will become DNase-free). APF filters from Millipore; grades C, D, E of glass fiber filters from Zefon; GF/D filter from Whatman; or a glass fiber filter from Sigma are all acceptable filters.

### *The system is leaking.*

System leaking does not automatically mean that the shearing assembly is clogged (although this is the most common reason for that). A (partial) clogging of the tubing connecting the shearing assembly to the system, or a broken syringe may cause the same effect. If microscopic observation showed that the shearing assembly is not clogged, then it is either the tubing, tubing connections, or the syringe. Disconnecting the shearing assembly (just the black box alone) and trying to wash the rest of the system with pre-filtered water in manual operation mode may help you clean the tubing. The first thing to check if the syringe is leaking, is the syringe itself. The next thing to check is that the syringe adapter is screwed into the valve as tightly as possible. If it's not tight enough, it will cause a leak there. If everything looks good, there might be something clogging the shearing assembly or the valve and the pressure will force the water back down. If the valve is suspected to be clogged, the HydroShear should be sent back to Digilab for repair.

### *My syringe is broken.*

The syringe used in HydroShear Plus unit is fortified and rarely gets broken, while the regular HydroShear has a standard syringe which is possible to break. The most common cause of this is clogging of the shearing assembly. Consult the text above for details on handling the situation.

If a syringe is broken and the shearing assembly is not clogged, the instrument (most often, syringe) is probably out of alignment. Consult syringe alignment procedure that comes with the manual. Contact our application specialist if you need further instructions.

### *Is my Hydroshear contaminated?*

The system is designed such that the metallic parts which are supposed to come into contact with wash buffers and the sample should not rust. The parts which are not supposed to be in contact with wash buffers may become rusty if they got exposed to acid (wash buffer 1) for a reasonably long time. If some metallic parts of the system look contaminated or rusty, it usually indicates that the system was not properly maintained in the past. To reduce the chances of rust or contamination, if a leak does occur, wipe liquid from the system as soon as possible. It is advisable to wash the place(s) affected by leakage with distilled water and dry with a filter paper.

*My sample is stuck in the syringe – how can I retrieve it without losing it?*

Refer to the Manual Operation in the software. In the manual mode, enter a positive volume number (for ex., 500) in the volume box, make sure valve is turned to input and input tubing is not touching any liquids, and click Start. Then enter a negative number (for ex., -500) in the volume box, make sure valve is turned to output, output tubing is in a vial for collection and click start. It usually takes 2-3 passes of these to make sure all of the liquid is expelled from the syringe.

*I want to retrieve as much of my sample as possible out of the system*

There is about 30-40 $\mu$ l of “dead volume” associated with the system. It combines the “dead volumes” of the syringe, tubing, and the shearing assembly. Due to this, some material may be lost when you eject the sample after completing the shearing procedure. In general, Digilab does not recommend using DNA sample in volumes smaller than 50 $\mu$ l. Please consider diluting the starting sample to at least 100 $\mu$ l volume before starting to shear (an option is to concentrate the sample back to the desired concentration after the shearing is completed). If sample dilution is not an option, a smaller sample volume can be used, but a portion of it will be lost inside the system (mostly, within the syringe). This won't cause cross contamination of you next sample if the recommended wash procedure is used. If complete recovery of a sample is essential, it is possible to get some of the sample out by filling the syringe up with air and expelling it out. In the manual operation, draw in 500 and expel -500 ( $\mu$ l). Two to four air passes may be sufficient. Unless it is critical to not dilute the sheared DNA sample try to wash the system with a small volume (~50 $\mu$ l) of pre-filtered buffer and collect the DNA fragments into the same tube before performing the air expelling steps. DNA then may be concentrated after using a standard precipitation procedure.

*The size of DNA fragments is different from what I expected.*

1. There is nothing on the gel after the DNA shearing is completed:
  - a) DNA concentration was too low to start with; concentrate it before separation on the gel;
  - b) initial DNA was of low molecular mass; always apply non-sheared DNA on the same gel as a positive control;
  - c) the wash procedure was not completed correctly, so the resulting pH in the system was highly acidic, or highly basic which destroyed DNA; always apply sufficient number of passes with the wash solution #3 (or a buffer); use excessive number of passes if water is used instead of wash solution #3; check the pH of the outgoing liquid before DNA sample is injected into the system;
  - d) one of the buffers/solutions was contaminated with nucleases; Digilab recommends using our wash solution kit in which every solution is certified to be nuclease free.
  
2. The size of the sheared DNA fragments is smaller/larger than I expected:
  - a) less than 20 shearing cycles (default value of the system) were applied in the procedure;
  - b) wrong shearing assembly was used; currently, Digilab supplies with shearing assemblies of three different sizes, each is recommended for generation of DNA fragments of specific size: small (0.85-3 kB), standard (1.4-8 kB), large (fragments from 4 kB and larger);
  - c) shearing assembly was used without pre-calibration; because each shearing assembly unit is unique and slightly different from each other, Digilab strongly recommends pre-calibrating of every new shearing assembly before use (consult the user Manual for details).

## Key Points

- Each new shearing assembly needs to be calibrated (instructions in manual);
- Only pre-filtered solutions should be used with the HydroShear; we recommend using our wash solutions kit (HSP00040) which is certified to be both nuclease-free, particle free and fully compatible with the system;
- DNA sample needs to be prepared/treated before shearing to contain no particles;
- Speed code 3 is the lowest (fastest speed) that the standard shearing unit can take;
- Speed code 6 is the lowest (fastest speed) that the small shearing unit can take;
  
- Some characteristics of the HydroShear Plus unit:
  - Dimensions: D 12" x W 5¼" x H 14¾" (footprint 8½")
  - Weight: 13.5 lbs (packaged 20 lbs)
  - Syringe (HSP00007) Max capacity 500 µl
  - Small assembly (HSP20002): Fragment size 850bp to 3kb – orifice size 0.0016"
  - Standard assembly (HSP20001): Fragment size 1.4kb to 8 kb – orifice size 0.0025"
  - Large assembly (HSP20003): Fragment size 4kb to >40kb – orifice size 0.0050"
  - Software version: v 2.1.1 compatible with Windows 98, 2000, NT, and XP
  
- Some characteristics of the Regular HydroShear unit:
  - Dimensions: D 12" x W 5¼" x H 12¾"
  - Weight: 9 lbs (packaged 16 lbs)
  - Syringe (JHSH010002) Max capacity 500 µl
  - Small assembly (JHSH204006): Fragment size 850bp to 3kb – orifice size 0.0016"
  - Standard assembly (JHSH204004): Fragment size 1.4kb to 8 kb – orifice size 0.0025"
  - Large assembly (JHSH204007): Fragment size 4kb to >40kb – orifice size 0.0050"
  - Software version: v 2.0.9 compatible with Windows 98, 2000, NT, and XP

General Information: <http://www.digilabglobal.com>

Contacts at Holliston, MA:

Orders: Sandra Morrison (508-893-3130 x 283; [smorrison@digilabglobal.com](mailto:smorrison@digilabglobal.com))  
Technical Questions: Dr. Igor Zlatkin (508-893-3130 x 252; [izlatkin@digilabglobal.com](mailto:izlatkin@digilabglobal.com))