

diagenode

A Hologic Company

Megaruptor[®] 2

DNA Shearing System



Guarantee

Limited one year global warranty

Diagenode guarantees all products from any manufacturing defects as we rigorously test all products to meet strict quality standards. Diagenode warrants that all standard components of its instruments will be free of defects in materials and workmanship, unless the original quotation or accompanying documentation states a different warranty period. All warranty periods begin on the date of delivery and apply only to the first purchaser of the product. If a manufacturing defect arises and a valid claim is received within the warranty period, Diagenode, at its discretion, will repair or replace the product in accordance with the warranty terms and conditions stated herein. In case of repair or replacement of a product under warranty, Diagenode will cover the expenses to return the repaired or replacement product.

This warranty covers only manufacturing defects and does not cover any damage caused by misuse, lack of compliance to recommendations stated in the manual, neglect, accidents, abrasion, or exposure to extreme temperatures, chemical solvents, or acids. We strongly recommend that maintenance or repairs of Diagenode's products are performed by our approved Diagenode service center. Improper or incorrectly performed maintenance or repairs will void the warranty.

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<https://www.diagenode.com/en/pages/support>

Looking for the manual of the previous version? Follow this link :

https://www.diagenode.com/files/products/shearing_technology/bioruptor/Bioruptor_pico_manual.pdf

Content

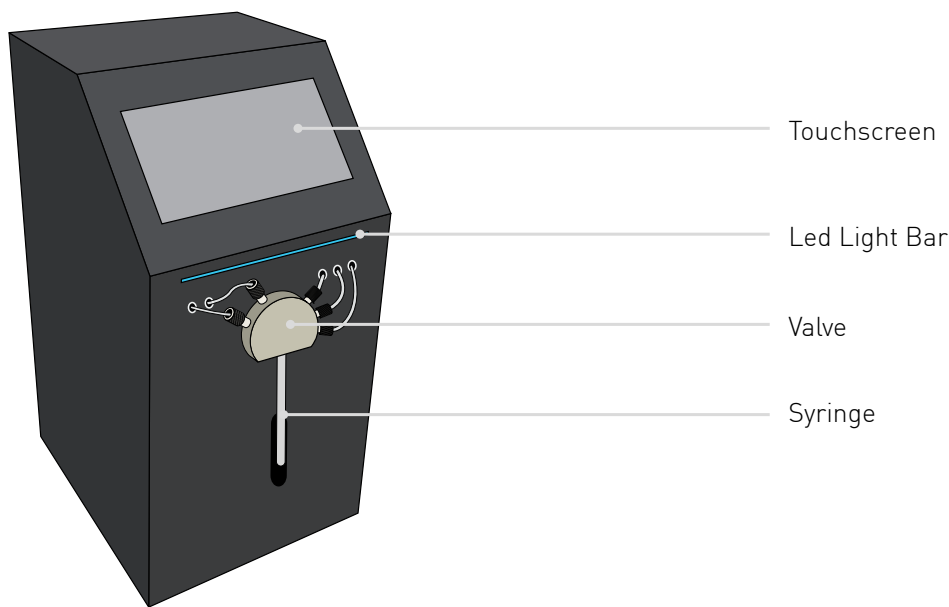
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General Information About the Megaruptor® 2

Congratulations on your purchase of the Megaruptor® 2 from Diagenode. The Megaruptor® 2 was designed to provide researchers with a simple, automated, and reproducible device for the fragmentation of DNA in the range of 3 kb - 75 kb. Shearing performance is independent of the source, concentration, temperature, or salt content of a DNA sample. Our user-friendly interface allows for two samples to be processed sequentially without additional user input and without cross-contamination. Just set the desired parameters and the automated system takes care of the rest. Clogging issues are eliminated in this design.

System Configuration and Components

The Megaruptor® 2 consists of an automated syringe pump with attached 9-port ceramic distribution valve and an integrated power supply. In order to control the device, a touchscreen interface is used. A 1mL syringe is provided. In order to fragment DNA, shearing consumables (Hydropores) must be attached to the valve.



Site Requirements

The Megaruptor® 2 requires access to a grounded 110V or 220V wall outlet. Different power cords are offered depending on your region. The female end of each power cord is identical and plugs into the power-input socket at the rear of the base unit. The male end of each power cord is different. You should have been supplied with a power cord suitable for the wall socket in your country or region.

The unit is designed for operating temperatures between 59° F (15° C) and 104° F (40° C), operating humidity between 20% and 80% relative humidity at 104° F, and storage temperatures between -4° F (20° C) and 149° F (65° C). Do not store, ship, or operate the device under conditions where temperature fluctuations could cause condensation within the unit.

System Performance

Principle

The Megaruptor® 2 uses the principle of mechanical shearing to fragment DNA. As DNA in solution is pumped through a Hydropore shearing device, it passes through an array of uniform pores. The resulting turbulent flow stretches and breaks the DNA strands. The length of the resulting fragments is dependent on the fluid flow rate and the size of the pores. Passage of the DNA molecules several times through the pores ensures that they will reach a minimum and uniform length as compared to a single pass through the Hydropore.

Fragment Length Range

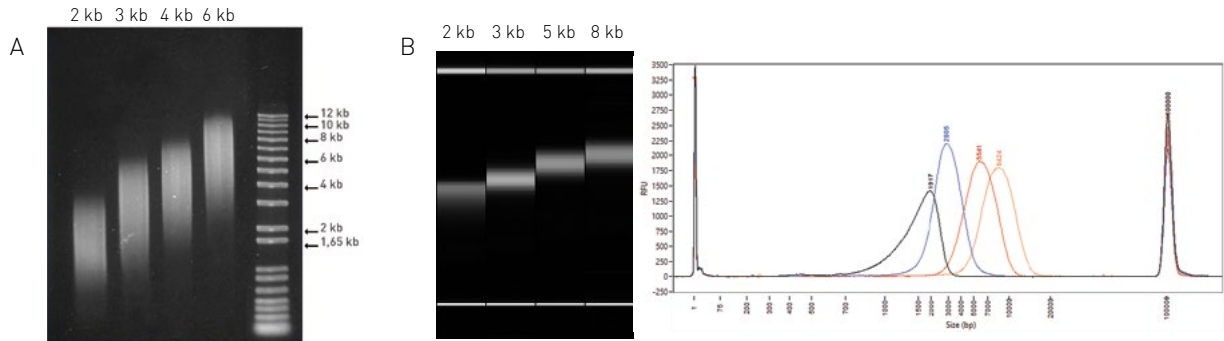
Two Hydropore shearing devices are available for use with the Megaruptor® 2. They each produce narrow distributions of fragments with the majority of molecules lying within a 2 to 3- fold size distribution. The Short Fragment Hydropore is capable of producing fragments with mean length of between 3 kb and 9 kb, while the Large Fragment Hydropore can produce fragments with mean length between 10 kb and 75 kb.

The user-friendly interface requires the operator to specify just two parameters: the volume of each sample, and desired mean fragment sizes. The Megaruptor® 2 translates this information into the appropriate operating conditions and processes the samples automatically.

Megaruptor® 2 Technical Specifications

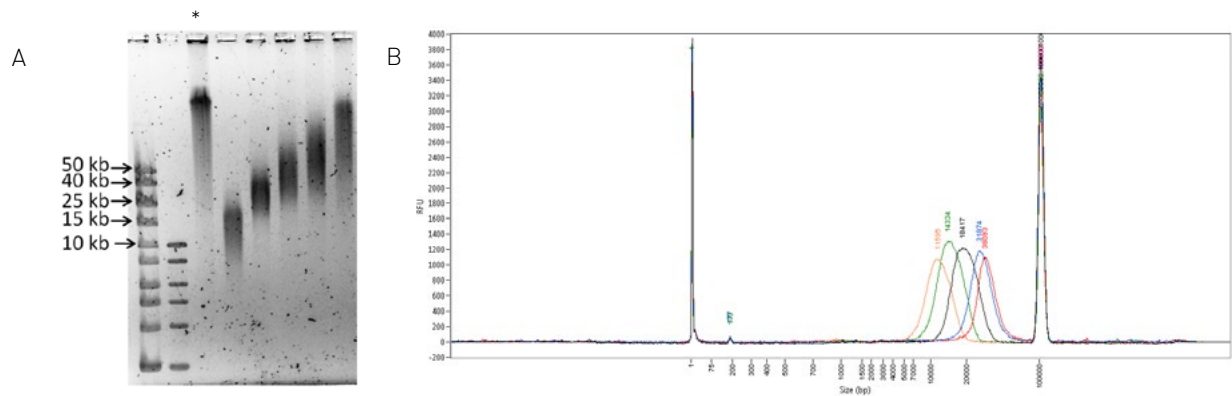
Megaruptor® 2	
Power Supply	24V, 3A (Internal)
Transformer Voltage Range: 90-264 VAC	Internal
Main Unit Dimensions	200(W) x 230(D) x 395(H) mm
Fragment Length Range Achievable	3 kb - 75 kb
Sample Volumes Accepted	Minimum: 50 µl. Maximum: 400 µl (depending on fragment length; see "Processing Samples" chapter) Recommended: 200 µl
Sample Concentration	0 - 50 ng/µl recommended: 25 ng/µl
Tubes	0.5 mL free-standing, provided
Throughput	Two samples processed in series
Time per sample	10 - 20 min (depending on target size); includes sample processing and system washing
Total weight	8,2 kg
User Interface	Touchscreen with Megaruptor® software

Reproducible and narrow size distribution



Reproducible and narrow DNA size distribution with Megaruptor® 2 using short fragment size Hydropores

Validation using two different DNA sources and two different methods of analysis. **A:** Shearing of lambda phage genomic DNA (20 ng/μl; 150 μl/sample) sheared at different speed settings and analyzed on 1% agarose gel. **B:** Fragment Analyzer profiles of human genomic DNA (25 ng/μl; 200 μl/sample) sheared at different software settings of 2 (contact us for this size), 3, 5 and 8 kb. (Standard Sensitivity Large Fragment Analysis Kit ; Advanced Analytical Technologies, Inc. was used for separation and fragment sizing).



Demonstrated shearing to fragment sizes between 10 kb and 75 kb with Megaruptor® 2 using long fragment size Hydropores

Image shows DNA size distribution of human genomic DNA sheared with long fragment Hydropores. **A:** DNA was analyzed by pulsed field gel electrophoresis (PFGE) in 1% agarose gel and a mean size of smears was estimated using Image Lab 4.1 software. **B:** Fragment Analyzer profiles of human genomic DNA (25 ng/μl; 200 μl/sample) sheared at different software settings of 10, 15, 20, 30 and 40 kb. (High Sensitivity Large Fragment Analysis Kit; Advanced Analytical Technologies, Inc. was used for separation and fragment sizing).

* indicates unsheared DNA

Installing Your System

Delivery Checklist

Ensure that all parts and materials have been delivered. Please report missing or damaged parts to your local sales representative.

Delivery Checklist for Megaruptor® 2

Description	Quantity
Power cable	1
Base unit	1
1 mL syringe , pre-installed	1
Bottles	5
System manual	1
Quick guide	1

Required reagents not supplied

- 0.5 M NaOH
- 0.5 M HCl
- H₂O (ultrapure)
- 10 mM TE or EB buffer

Note: All reagents should be of molecular grade. It is mandatory to filter all solutions through a 0.22 µm PES filter. (Pall Life Sciences AVFP02M or Corning EK-680502). It's possible to use other 0.22 µm filter references on condition that they are compatible with HCl and NaOH solutions.

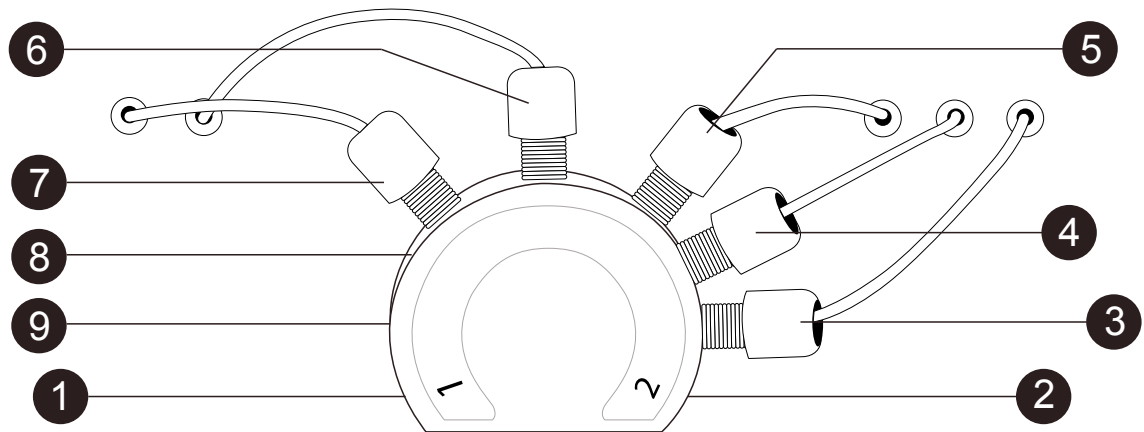
Connections and Communication

1. Plug the female end of the power cord into the AC power receptacle at the back of the base unit. Plug the 3-prong male end into a grounded wall outlet.
2. Make sure that the syringe is tightly connected to the valve. Turn it clockwise (text facing the user should move from left to right) until it no longer moves. Tools are not necessary.

Reagent Set-Up

The Megaruptor® 2 contains a ceramic valve with 9 ports for the automation of shearing and washing steps. It is imperative that the wash reagents (not supplied) and samples are connected to the correct ports on the valve. The default set-up is as follows:

Orientation of ports on the ceramic valve



1. **Sample 1.** See the section on Processing Samples for instructions on preparing the sample for shearing and setting the shearing parameters in the software.
- 2: **Sample 2.** See the section on Processing Samples for instructions on preparing the sample for shearing and setting the shearing parameters in the software.
3. **TE Buffer.** Place the end of the tubing into a reservoir of 10 mM TE Buffer. If it is imperative that EDTA is not introduced into the sample, the TE buffer can be substituted with 10 mM Tris-Cl, pH 7.5. It is mandatory that all wash solutions are filtered to 0.22 μm before use.
4. **H₂O.** Place the end of the tubing into a reservoir of H₂O. It is mandatory that all wash solutions are filtered to 0.22 μm before use.
5. **NaOH.** Place the end of the tubing into a reservoir of 0.5 M NaOH. It is mandatory that all wash solutions are filtered to 0.22 μm before use.
6. **HCl.** Place the end of the tubing into a reservoir of 0.5 M HCl. It is mandatory that all wash solutions are filtered to 0.22 μm before use.
7. **Waste.** Place the end of the tubing into a waste receptacle with volume at least 100 mL.
8. **Air.**
9. **Unused.**

Note: When choosing the volume of wash reagent to prepare, be aware that washing after each sample will consume approximately 1.5 mL of HCl, NaOH, and H₂O, and approximately 4 mL of TE. The 250 mL bottles which have been supplied should be specific to each solution.

Processing Samples

Sample Preparation

While the design of the Hydropore enables the Megaruptor® 2 to fragment DNA without the clogging problems encountered in single-orifice devices, it is still important that the sample be pure DNA at a reasonably low concentration. These guidelines will assist the user in preparing a sample that will be quickly and easily sheared with the Megaruptor® 2.

We highly recommend to control the integrity and the size of original DNA using an electrophoresis instrument (ex: Fragment Analyser). The fragmentation performance is largely independent of standard buffer components and is tolerant of a wide range of salt concentrations and pH. However, often during the purification process and particularly with plant sources, detergents such as SDS, CTAB, or Skarkosyl are used. If detergent is present during the shearing process, the sample will become frothy. The presence of air in the sample, as well as the unexpected increase in sample volume, will both be detrimental to the shearing performance.

Samples should be RNase treated before the shearing. The presence of RNA in samples can result on a less regular shearing.

If any detergent or suspended particles are visible in the sample, it is best to **centrifuge to remove these impurities from the DNA in solution** in order to avoid clogging the Hydropore. Spin the samples at room temperature for 15 minutes at 16,000 x g. Remove the supernatant containing the DNA sample, leaving the pellet undisturbed. If it is possible that the DNA itself has precipitated, use whatever heating or stirring measures necessary to dissolve the nucleic acids prior to spinning down so that the DNA is not lost.

In principle, the Megaruptor® 2 is capable of shearing DNA solutions regardless of the DNA concentration. However, the viscosity of fluids containing high concentrations of DNA, particularly very high molecular weight genomic DNA, can make the process problematic. Highly viscous fluid will move through the tubing and Hydropore more slowly than solutions with lower concentrations of DNA. This makes the fluid flow unpredictable and difficult to efficiently automate. Highly concentrated genomic DNA also has a tendency to aggregate and clump. This can be attenuated by mixing the sample via rapid pipetting.

Diagenode recommends that the concentration of DNA shouldn't be higher than 50 µg/mL for optimal performance. Diagenode suggests that high concentration samples can be diluted in TE buffer down to 50 µg/mL prior to processing. Additionally, the viscosity of DNA solutions containing very high molecular weight genomic DNA can be slightly reduced by rapidly pipetting the sample to begin the fragmentation process before submitting the sample to the Megaruptor® 2. This is not recommended for samples to be sheared to a size higher than 30 kb

The Megaruptor® 2 is designed to process sample volumes between 50 µl and 400 µl. It is recommended to use a minimum sample volume of 100 - 250 µl for 3 kb fragments. If the sample volume exceeds this range after dilution, it can be split into two or more aliquots for shearing. In this case, a new Hydropore is not necessary. Simply reuse the same Hydropore for each subsequent aliquot of the same sample. The number of times a Hydropore can be reused depends on the purity of the sample being sheared. Process the next aliquot immediately after the end of the previous one. The hydropore should not dry.

Shearing small volume samples (<75 µl) at high speeds (targeting fragment sizes 3 kb) can lead to larger-than-expected fragments. This is likely due to small bubbles decreasing the effective flow rate, and the pump having difficulty accelerating to the required speed over very short stroke lengths. The user should be aware of this possibility.

System Operation

Interface



Shearing : Running a shearing experiment (follow instructions)



Settings : Visualize and change the parameters of the Megaruptor® 2



Time : Display the current time and date



Info : General information about your Megaruptor® 2



Wash : Sequence for washing the instrument

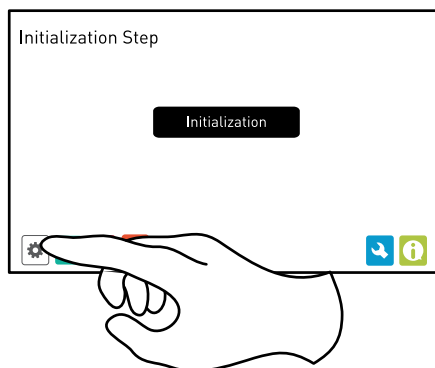


Initialization : Initialize the pump and set the syringe to its "0" position

LED LIGHT BAR

- The LED light bar allows the tracking of the processing of your samples.
- The **green light** indicating the progression.
- The **red light** indicates an error.
- The **blue light** means the system is waiting for the operator.
- The **pink light** flashes when the pump is initializing.

Initialize

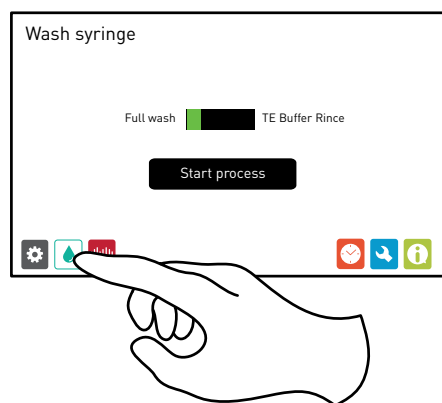


The pump must be initialized each time it is turned on. If the pump has been left on since its last use, it is not necessary to re-initialize. The pump must be re-initialized each time a new syringe is installed.

Click the "Initialization" button. The syringe plunger will move down briefly and then back up until it hits the valve. This sets the syringe to its "0" position.

Wash

Two wash options are available in the software: "Full Wash" & "TE buffer Rinse". The syringe and valve must be thoroughly washed and rinsed between each experiment. While the software will automatically wash the pump after each sample is processed, it is advisable to also wash the system before the first sample is sheared. Therefore, it's highly recommended to run a "Full Wash" before and after the shearing.

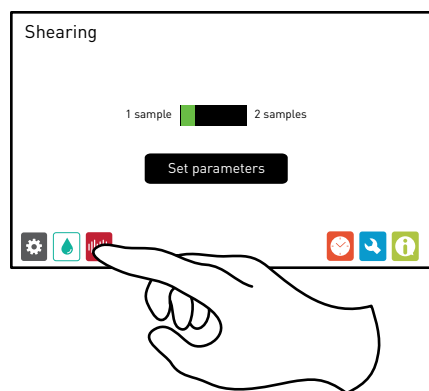


Select “Full Wash” option in the wash syringe menu then click “Start”. The pump will automatically rinse the syringe and valve with four solutions in order to eliminate any potential crossover contamination from DNA in the previous sample. First, 0.5 M HCl is used to depurinate the DNA, eliminating the purines from the DNA strand and also causing DNA strand breakage. Next 0.5 M NaOH is introduced to denature the DNA. Following the NaOH wash, any residual DNA will be short single-stranded fragments of pyrimidines. These are washed away with several rinses with water, before a series of washes with TE buffer make the syringe and valve DNA-friendly and ready for the next sample.

The “Stop” button can be used to interrupt the wash sequence at any time. However, this is not recommended, as prematurely stopping the wash cycle may leave HCl or NaOH in the syringe and valve, causing the destruction of any DNA sample introduced at a later time. If the wash cycle must be stopped, it is recommended that a subsequent full wash be completed before processing any samples.

Note: If the “TE rinse” option has been selected, an abbreviated Wash protocol will be used. This option can be selected only when samples attached in pore 1 and 2 are both aliquots from the same sample. “TE buffer Rinse” will not avoid crossover contamination.

Select Number of Samples

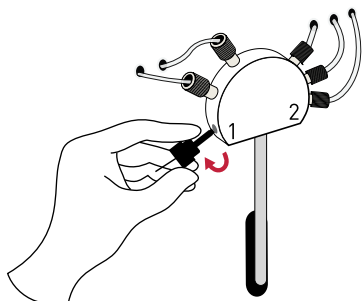


The Megaruptor® 2 can process two samples consecutively without additional user input. Enter the number of samples and set the parameters.

Enter Parameters

For each sample, select the type of hydropore (white = short 3-9 kb / black = long 10-75 kb) and enter the volume of the DNA solution. See the section on Sample Preparation (page 9) when deciding what sample volume and concentration to use. Next, choose the mean fragment size that is desired for each sample. When using the Short Fragment Hydropore, the menu will contain a range of choices between 3 kb and 9 kb. If the Large Fragment Hydropore is selected, the user can select from mean fragment sizes of 10 kb - 75 kb.

Install Hydropores



Securely fix the hydropore at position 1 and, if two samples selected, 2 before you preload the hydropore. Make sure the hydropore is correctly inserted in the internal screw thread of the pore before tightening. **At this step, the hydropore must be screwed very hard.**

Pre-Load Hydropores

This option minimizes the air in the system, leading to decreased sample splashing and more consistent results. Hydropore pre-loading is included in all runs.

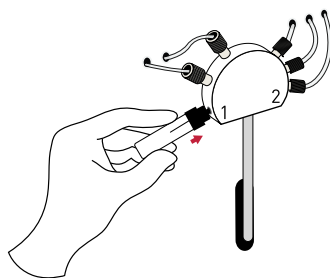
Note: This procedure will dilute your sample by 45 μl .

Click the “Pre-Load Hydropores” button. A prompt appears for the user to attach Hydropores to appropriate ports on the valve.

Note: For this step, it is very important that the Hydropores are securely threaded into the valve but the samples are NOT attached to the Hydropores!

After attaching the Hydropores, click the “Start Preloading” button. The pump will prime the Hydropores with TE buffer. A small drop will come out from the hydropore tubing. If the drop fall down, this will not affect the shearing process but will slightly modify the expected sample concentration. If no drop is observed during the Pre-load step, the Pre-load may be not performed correctly. In this case, perform a TE buffer rinse and restart again the shearing.

Install Samples



Gently press the sample tube into the “lid” of the Hydropore. It should be held securely. It may be helpful to remove the attached tube cap.

The tip of the tubing can be about 1-2 mm from the bottom of the tube and the entirety of the sample will still be drawn into the syringe.

Monitor Progress

When the samples are secured in their proper positions, click “Start” to begin the shearing sequence.

The “Instrument Status” text box will indicate the status of each sample. Additionally, progress bars are provided to help monitor the shearing.

When using very long genomic DNA, the DNA solution can be quite viscous. Pumping viscous DNA at high speeds

does not lead to optimal performance. Thereby, by default, when the user selects a mean fragment size of 5 kb or smaller, the DNA is first sheared at a lower speed before the normal shear cycle begins. This starts to break up the DNA so that the solution is less viscous. This is called the "Pre-Shear".

Thus, the steps involved in the processing of each sample are as follows:

1. **Sample Load** – The sample is slowly drawn into the syringe and then ejected back into the sample tube 3 times. This serves to mix the sample with the TE in the Hydropore and helps to eliminate any air trapped in the fluid path.
2. **Pre-Shear (less than 5 kb)** – The sample is cycled through the Hydropore seven times at a slow flow rate.
3. **Shear** – The sample is cycled through the Hydropore 15 times at a speed that is determined by the desired final fragment length. Shorter fragment lengths require the sample to be pumped at higher flow rates.
4. **Sample Recovery** – At the conclusion of the shear step, some of the sample will be left in the tubing attached to the Hydropore. In order to recover this portion of the sample, the syringe will draw in air and expel it through the shearing device tubing. When this step is complete, the sample has been fully processed and can be removed for downstream reactions.
5. **Wash** – The pump will now wash the syringe and valve as described in the "Wash" section to prepare the device for the next sample.

The "Stop" button may be used to interrupt a shear in progress. This will immediately stop all pump movements. Note that when the Stop button is used, it is not possible to resume the program from the point at which it was stopped. After you click on the stop button, the system will direct you to the settings page where the sample can be recovered using the button "Recover sample".

The "Pause" button allows the user to temporarily halt the program. When the Pause button is used, the pump will finish its current movement before stopping. Clicking the Pause button again will allow the program to continue operating from the point at which it was halted.

Analysis of sheared samples

The sheared samples can be analysed using any instrument that can be appropriate for the size of generated fragments. For example, we recommend the following:

- For fragments below 5 kb, the Fragment Analyser (Advanced Analytical) or an agarose gel with a suitable ladder can be used
- For fragments up to 50 kb, a Pulsed field gel electrophoresis instrument (PFGE) or the Fragment Analyser (Advanced Analytical) combined with the High Sensitivity Large Fragment 50kb kit can be used.
- For fragments above 50 kb, a Pulsed field gel electrophoresis instrument (PFGE) can be used.

Note: When using the High Sensitivity Large Fragment 50 kb kit (Advanced Analytical), we highly recommend to use no more than 1 ng of sheared DNA for the sizing. Using more than 1 ng can result on a less consistent sizing.

We highly recommend to analyse in parallel sheared DNA and original DNA (not sheared).

Troubleshooting FAQ

Q: The volume of DNA sample appears to be decreasing.

A: Look for signs of wetness or dripping at the connections between the syringe and valve and between the Hydropore and valve. Check and tighten all fluidic connections.

Check to see if the sample has splashed up onto the walls of the sample tube. If it has press pause. Remove the tube from the Hydropore. Tap the tube to make the drops slide back to the bottom of the tube.

Q: How are the volume and concentration of the sample affected by the shearing process?

A: If the "Pre-load Hydropore" box in the Setup Device tab is checked, as is recommended and is the default, each sample will be diluted by about 45 μ l of TE.

Q: Bubbles are visible in the syringe during shearing.

A: It is normal for small bubbles to appear when the pump is aspirating the sample into the syringe. These bubbles typically cling to the walls of the syringe or to the Teflon seal on the plunger. When the plunger has completed its downward movement, the pressure in the syringe will equilibrate and the bubbles should decrease in size and be barely visible. They will be further compressed when the fluid is ejected out of the syringe. These small bubbles do not affect shearing performance.

If larger bubbles are present in the syringe and they do not disappear when the aspiration step is completed, it is possible that shearing performance will be affected. Typically this is caused by a dirty syringe or loose connections. Check and tighten all fluidic connections and the syringe connection to the valve. Perform several full washes. If none of these measures are successful the seal on the syringe may have been compromised and the syringe should be replaced.

Q: During the sample load step and subsequent shearing steps, the sample is not being fully drawn into the syringe.

A: This is most likely caused by a sample with DNA concentration in excess of 50 μ g/mL. The sample is too viscous to be drawn into the syringe in the time allotted. In some cases, shearing a sample with very high concentration multiple times will result in satisfactory performance. However, Diagenode recommends diluting samples to 50 μ g/mL.

This may also be due to a dirty or contaminated sample causing the Hydropore to clog. A sample with many suspended particles, proteins, or cellular debris can clog the Hydropore. See the section on "Sample Preparation" for guidelines.

Q: The DNA fragments are not at the expected size.

A: The software is calibrated to consistently provide reproducible results regardless of sample conditions. One exception is shearing small volumes (less than 75 μ l) at high speeds (3 kb). It is due to small bubbles having a greater effect on small volumes or the pump being unable to achieve high speeds over short distances. Also, check the size and the purity of starting DNA. Not pure and/or fragmented DNA will result on a less consistent shearing.

Q: The sample appears to have been lost/There is no DNA on the Bioanalyzer trace/There is no DNA on the agarose gel.

A: The most likely cause is that the sample has been contaminated with HCl from the wash buffer. Check closely to make sure that each reagent line is connected to the correct port on the valve. Also verify that the reagent bottles are NOT placed at a higher elevation than the device itself.

Q: A power failure occurred or Megaruptor was switched off by error during the shearing process.

A: Switch ON the Megaruptor, an error message will ask you to click on the area below the error message. This will direct you to the settings page where you can recover the sample using the "Recover sample" button. The system will detect automatically if the sample 1 or the sample 2 should be recovered. The LED light will be green after the sample will be correctly recovered to the hydrotube.

Maintenance

While the pump is in regular use, additional maintenance steps are typically unnecessary. The syringe wash that is performed after each sample should sufficiently flush the fluid paths to keep them clean and clear. However, steps can be taken to optimize the performance and lifetime of the pump and syringe:

- Perform 3 to 5 full washes successively each month
- If the Megaruptor 2 will not be used for a long period, make a full wash, empty the buffer bottles and rinse them with Ultrapure water then re-install them in the Megaruptor
- If the buffers date from more than 3 months, replace them with new filtered ones

Installing a Syringe

The Megaruptor® 2 comes with a 1 mL syringe pre-installed. If the syringe becomes dirty or the syringe fails, it is possible to replace it. To install a syringe, follow the steps below.

1. Initialize the pump. Next, switch to the Operation tab and click on the “Exchange Syringe” button under the heading “Load Position”. This will lower the syringe plunger.
2. Remove the plunger lock screw.
3. Screw the syringe into the common port located on the underside of the valve. The syringe should be “finger tight”. The use of tools is unnecessary and may cause damage to the syringe or valve.
4. Pull the syringe plunger down to the plunger holder assembly.
5. Align the plunger button through hole to the carriage mounting hole.
6. Slide the plunger lock screw through the plunger button and fasten to the carriage.
7. Be sure to re-initialize on the Home tab after the new syringe is installed.



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