diagenode

Innovating Epigenetics Solutions

Megaruptor® 3

DNA Shearing System

USER GUIDE

Version 1 03_2019

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 for a period of one (1) year from the date that the warranty period begins, 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.

Technical assistance & ordering information

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

Contents

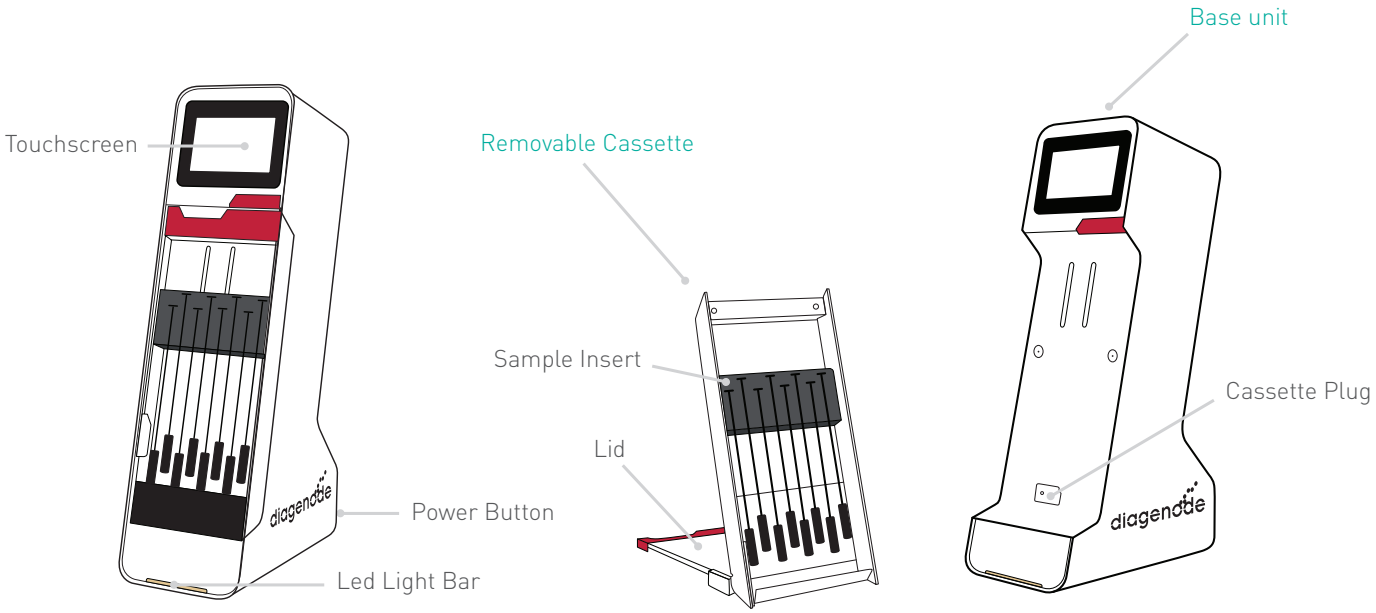
General Information About the Megaruptor® 3

Congratulations on your purchase of the Megaruptor 3 from Diagenode.

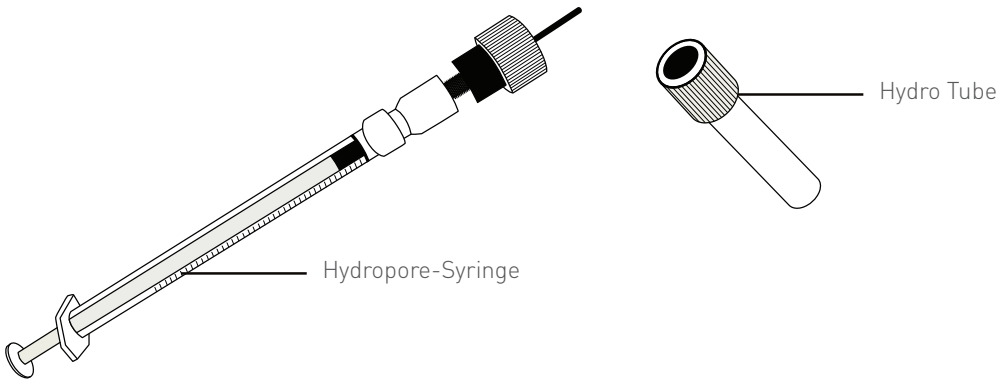
The Megaruptor 3 was designed to provide researchers with a simple, high throughput, and reproducible solution for the fragmentation of DNA in the range between 5 kb and higher than 100 kb. Our user-friendly interface allows for up to 8 samples to be processed simultaneously without additional user input. The removable cassette makes the system flexible and ergonomic. Just set the desired parameters and the Megaruptor takes care of the rest quietly and efficiently.

System Configuration & Components

The Megaruptor 3 consists of a base unit and a removable cassette. In order to control the device, a touchscreen interface is used. In order to fragment DNA, shearing consumables must be correctly installed on the cassette.



As for the shearing consumables, ready to use assemblies of Hydropore-Syringe and Hydro Tubes are provided separately (Megaruptor® 3 Shearing Kit – Cat. No. E07010003).



Site Requirements

The Megaruptor 3 requires access to a grounded 100V or 240V wall outlet. Different power cords are offered depending on the region. 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 (40°C), and storage temperatures between -4° F (20° C) and 122° F (50° 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 3 is an automated system that controls the liquid flow at the level of a precisely manufactured consumable: the Hydropore (same chore technology as our previous models, the Megaruptor® 1 and the Megaruptor® 2). It uses the principle of mechanical shearing to fragment DNA. As DNA in solution is pushed 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 mainly on the fluid flow rate. Passage of the DNA molecules several times through the pores ensures a minimum and uniform fragment length.

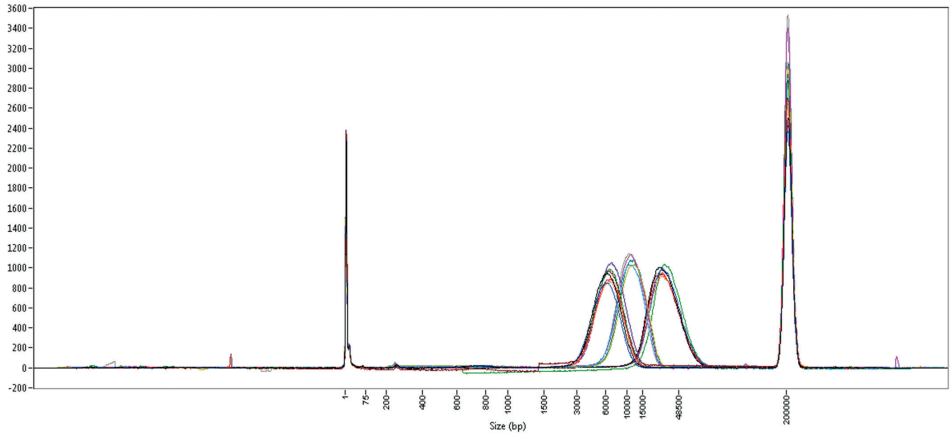
Fragment Length Range

Shearing consumables are available for use with the Megaruptor 3 (Megaruptor® 3 Shearing Kit – Cat. No. E07010003) and are capable of producing fragments with an average size between 5 kb and more than 100 kb with a DNA shearing variability ‹ 15% CV (if same batch of sample and same condition of shearing and analysis).

The user-friendly interface requires the operator to specify few parameters: the concentration and the volume of the sample(s) and the speed of the system. The Megaruptor 3 automatically translates these parameters into the operating conditions to the sample characteristics and the target size range.

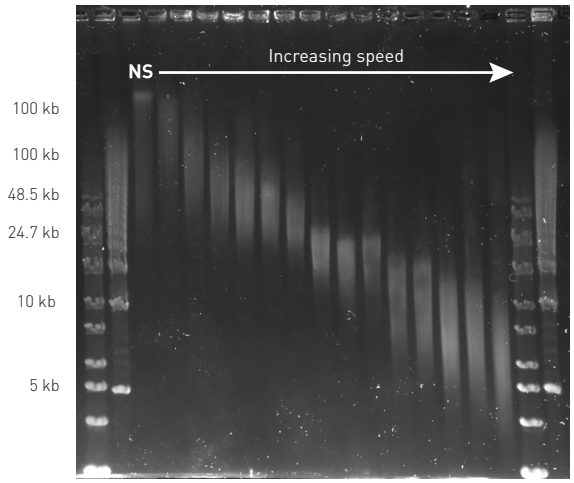
Reproducible & Narrow Size Distribution

A. Reproducibility and size distribution



A: Fragment Analyzer profiles of human genomic DNA samples (25 ng/µl; 200 µl/sample) sheared to 6, 10 and 30 kb.

B. Versatile fragment range



B: DNA samples sheared at different speed settings were analyzed by Pulsed Field Gel Electrophoresis (PFGE) in 1% agarose gel. *(NS: Not Sheared).*

Technical Specifications

External Power Supply Unit Voltage Range	100V AC – 240V AC 50Hz/60Hz
Electrical Consumption Maximum	90W Max
Main Unit Dimensions	470 (W) x 140 (D) x 25 (H) mm
Total Weight	6 kg with Cassette included (0,8kg)
Fragment Length Range Achievable	5 kb - ›100 kb
Sample Volume Range	65-500 µl
Sample Concentration Range	0-150 ng/µl
Throughput	1 to 8 samples in parallel
Time per Run	Variable: depending on sample volume, concentration, and target size
Shearing Consumables	Megaruptor® 3 Shearing Kit (16 samples) - Cat. No. E07010003
User Interface	Touchscreen with Megaruptor® software

Installing Your System

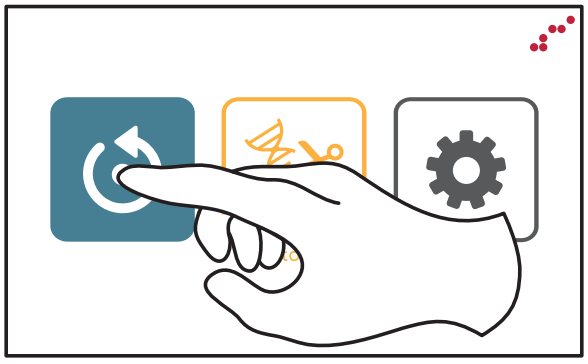
Delivery Checklist

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

Delivery Checklist for Megaruptor 3	
Description	**Quantity**
Power cable	1
Power supply	1
Base unit	1
Cassette	1
Quick guide	1

Connections & Communication

1. Plug the female end of the power cable into the power receptacle of the power supply. Plug the male end of the power supply at the back of the base unit. Finally, plug the male end of the power cable into a grounded wall outlet.
2. Install the cassette on the base unit.
3. Start the system by pressing the power button at the back. The led indicator lights up in blue.
4. Initialize the system. The initialization step must be performed if the icon of the top menu is flashing.



***CAUTION**: Always turn off the Megaruptor 3 after use or at the end of the day using the power button at the back.*

Processing Samples

Sample Preparation

Sample concentration

The Megaruptor 3 is capable of shearing DNA solutions in the concentration range of 0-150 ng/µl. For concentration up to 50 ng/µl, the standard protocol is applied. However, for DNA samples for which the concentration is higher than 50 ng/µl, the viscosity may be higher and the samples will move through the Hydropores more slowly. For that reason, the standard shearing protocol has been adapted for highly concentrated samples (for more details see following sections). Depending on the concentration entered into the system, the Megaruptor 3 will automatically adapt the protocol to ensure a better efficiency. The High Concentration protocol leads to longer run times and it requires sample volume higher than 200 µl.

The table below summarizes the different points mentioned above:

Concentration range	Comments
0 – 150 ng/µl	Sample Concentration Range
0 – 50 ng/µl	Sample Concentration Range for **Standard Protocol**
51 – 150 ng/µl	Sample Concentration Range for **High Concentration Protocol** (minimum 200 µl of sample volume*)

** For conditions different from the table above, please contact Diagenode for more assistance.*
https://www.diagenode.com/en/pages/support

> ***CAUTION**: Samples ran in parallel in the Megaruptor 3 can have slightly different concentration (+/- 25 ng/µl) without affecting the shearing performances. However, all the samples should be within the same concentration range (ex: don't run in the same time standard samples and highly concentrated samples).*

Sample volume

The Megaruptor 3 is optimized to process sample volumes between 65 µl and 500 µl. Please note that samples for which the volumes are lower than

100 µl may result in a progressively less efficient shearing due to a less efficient mixing (smaller is the volume, less efficient is the shearing). Thus, for sample volume below 100 µl, more optimization is required. However, a protocol for a sample volume of 65 µl has been already optimized for a perfect match with low volume PacBio® and Oxford™ Nanopore library prep.

The table below summarizes the different points mentioned above:

Volume range	Comments
65 – 500 µl	Sample Volume Range
100 – 500 µl & 65 µl	Recommended Sample Volumes
100 – 500 µl	Sample Volume Range for **Standard Protocol**
65 µl	Sample Volume for **Low Volume Protocol**

***CAUTION**: Samples ran in parallel in the Megaruptor 3 should have the same volume to ensure efficient and reproducible shearing results.*

***NOTE**: Please be aware that a dead volume between 5 and 15 µl should be considered due to plastic/surface retention and pipetting errors.*

For sample volumes out of the recommended range, please contact Diagenode for more assistance:
https://www.diagenode.com/en/pages/support

Sample viscosity, purity, and integrity

The Hydropores have been designed to limit the clogging problems encountered in single-orifice devices. It is nevertheless important to keep the sample as pure as possible with an appropriate fluidity (the sample should keep its liquid aspect) to be able to pass through the Hydropores optimally.

Viscosity

Viscous samples (thick or gelly aspect) may be due to several reasons. Highly concentrated samples and/or very high molecular weight genomic DNA trend to aggregate and clump. Also, the presence of detergents, specific reagents and impurities will increase the viscosity.

The viscosity of DNA solutions can be slightly reduced by:

- Mixing the samples via rapid pipetting before submitting them to the Megaruptor 3 (not recommended if the target size after shearing is higher than 20 kb) and/or;
- Diluting the samples 2 or 4 folds using TE buffer prior processing.

***NOTE**: In case of highly viscous samples, stay close to the system during the first steps to be sure that the samples are passing correctly through the Hydropores to the syringe (movement up) or to the Hydro Tubes (movement down).*

Purity

The fragmentation performance is largely independent of standard buffer components and is tolerant to a wide range of salt concentrations and pH. However, during the purification process and particularly with plant sources, detergents such as SDS, CTAB, or Sarkosyl are often used. If detergent is present during the shearing process, the sample may become foamy and could negatively affect the shearing performance. If any detergent or suspended particles are suspected or visible in the sample, it is better to centrifuge to remove these impurities from the DNA in solution. To do so,

1. Spin the samples at room temperature for 15 minutes at 16,000 x g.
2. Remove the supernatant containing the DNA sample, leaving the pellet undisturbed. In order to minimize the DNA precipitation, use whatever heating or stirring measures necessary to dissolve the nucleic acids prior to spinning down.

Integrity

We highly recommend to control the integrity and the size of original DNA using an electrophoresis instrument (ex: Fragment Analyzer) to be sure the samples are not degraded.

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

System Operation

Interface

Initialization: Set the system to its "0" position

Protocols: Run a shearing experiment (follow instructions)

Go & Shear: Start a new shearing protocol

User protocols: Record your protocol

Settings: Visualize and change the parameters of the Megaruptor 3

Sound & Screen: Visualize and change the parameters of the sound and the screen

Information: General information about your Megaruptor 3

Advanced Mode: Access for administrators

Modification: Modify the parameters

Led Light Bar

The LED light bar allows the tracking of the processing of your samples.

The **blue light** means the system is waiting for the operator and is flashing if the system needs to be initialized.

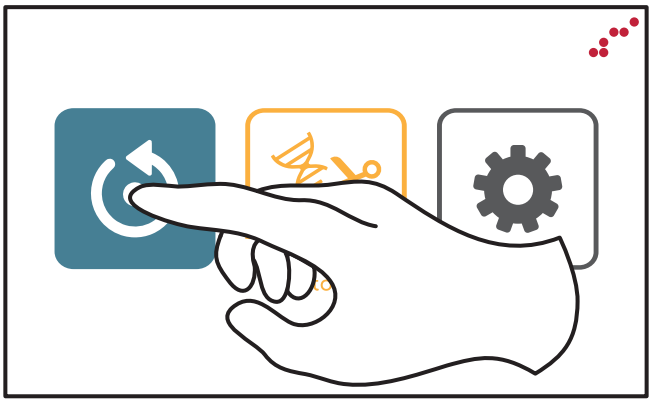
The **green light** indicates the progression of the run.

The **orange light** means the protocol is on pause.

The **red light** indicates an alert message.

Initialize

The system must be initialized each time it is turned on. Click on the blinking icon of the top menu.

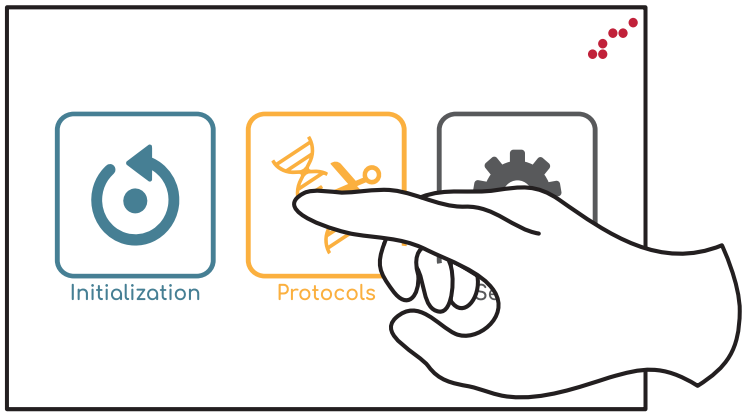


- If the cassette is **already installed** on the base unit, as indicated on the screen, remove the cassette by shifting it up and wait 3 s. A brief movement (down then up) of the 2 metallic arms will set the device at its zero position.
- If the cassette is **not yet installed** on the base unit, the initialization step will be done 3 s after clicking on the initialization icon.

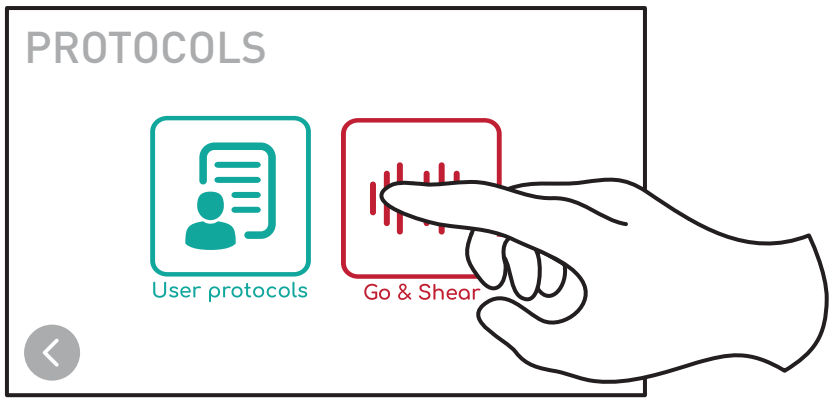
If the initialization step was not done when switching ON the system, the Megaruptor will invite the user to initialize the system before starting any shearing protocol.

Start Shearing Experiment

1. Click on 'Protocols' icon of the top menu.



2. Click on 'Go & Shear' icon to start a new protocol or click on 'User protocols' icon to find your pre-recorded shearing protocol.



Enter Parameters

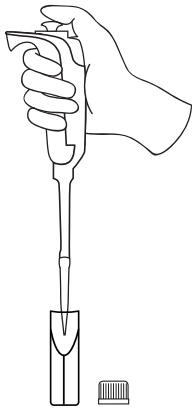
3. Enter the sample concentration (please refer to the Sample Preparation section page 10).
4. Enter the sample volume (please refer to the Sample Preparation section page 10).
5. Enter the speed value (please refer to the Shearing Protocols section page 22)

The Megaruptor 3 will display a summary of the entered shearing parameters before starting the shearing protocol.

> ***NOTE**: The Megaruptor 3 will automatically save the parameters from the previous run even after switching OFF the instrument. Please double check the shearing parameters each time you start a new run in case of multiple users of the device.*

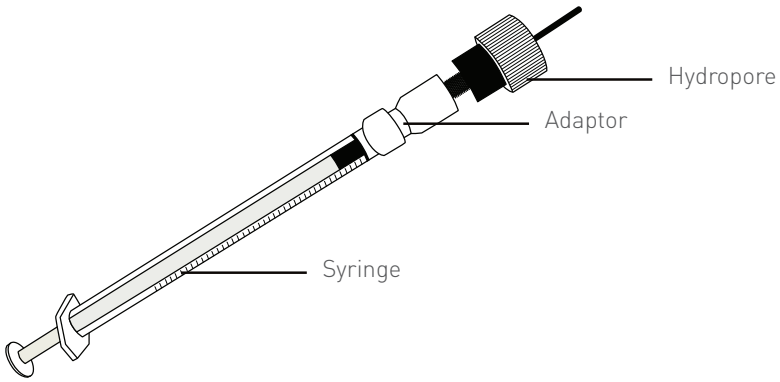
Prepare Consumables & Samples

6. Transfer the samples to the Hydro Tubes.



7. Unpack the assemblies of Hydropore-Syringe. Make sure that the plunger of the syringe is completely compressed and thus eject any remaining air out of the syringe before moving to the next step.

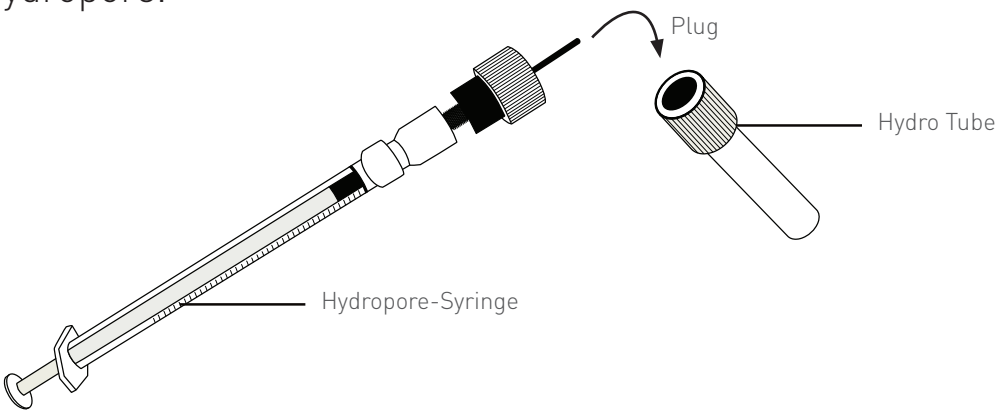
8. Be sure that all 3 parts (Syringe + Adaptor + Hydropore) are correctly tightened together.



***NOTE**: The assemblies are ready to use, however they may be slightly loose due to transportation.*

***NOTE**: In some cases, the assemblies are slightly inclined. This will not affect the shearing performances as long as the assemblies are well tightened.*

9. Hold the Hydropore Syringe by the Hydropore and plug the Hydro Tube into the Hydropore carefully. There is no screwing needed but make sure the Hydro Tube is well inserted into the Hydropore.



Watch the video on our Diagenode Youtube channel
How to set up your sample(s)

Install Samples

10. Place the samples in the sample insert when the cassette is on the base unit of the Megaruptor 3. For more flexibility, the samples can also be inserted when the cassette is out of its base unit.

- If the cassette is **already on its base unit**:

1. Open the lid of the cassette;
2. Install the samples from the bottom to the top:

    a. Install first the Hydro Tube and the Hydropore in the lower part of the sample insert of the cassette;

    b. Install the main body of the syringe in the intermediate part of the sample insert. Simultaneously, place carefully the plunger of the syringe in the upper part of the sample insert (movable part of the insert).

3. Make sure all consumables are correctly inserted into the cassette;

4. Close the lid of the cassette.

- If the cassette is **out of its base unit**:

1. Open the lid of the cassette;
2. Install the samples from the bottom to the top:

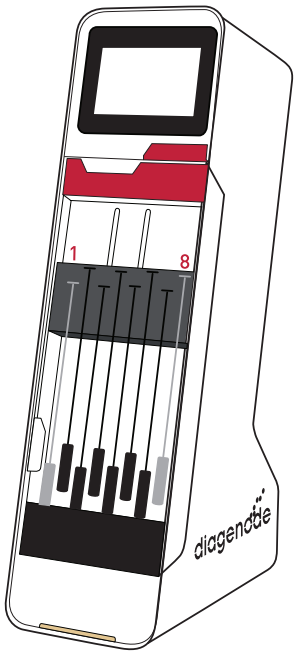
    a. Install first the Hydro Tube and the Hydropore in the lower part of the sample insert of the cassette;

    b. Install the main body of the syringe in the intermediate part of the sample insert. Adjust simultaneously and carefully the position of the upper part of the insert (movable part of the insert) to be able to insert the plunger of the syringe.

***NOTE**: Never pull the plunger of the Syringe, this may shear the sample inconsistently.*

3. Make sure all consumables are correctly inserted into the cassette;
4. Close the lid of the cassette;
5. Place the cassette on its base unit.

- **Always make sure that the initialization step is accomplished before installing the samples** (see initialization step section page 14).
- **To ensure an optimal functioning, always set consumables at each extremity (position 1 and position 8) of the cassette in order to balance the system**. The consumables used for balancing the cassette can contain the samples or can be empty. In case of 1 sample to be processed, an empty Hydopore-Syringe + an empty tube can be used as a balance and can be reused later (if new).



Start Processing & Monitor Progress

Once the samples are secured in their proper positions, the cassette is installed on the instrument, and the shearing parameters are entered:

11. Click "Go" to start the shearing process. A progress bar, the remaining time, and the name of each step of the protocol (described below) are displayed on the screen. The led indicator lights up in green.

Description of the shearing process

Step 1: Preload

During this step, a small volume is aspirated at minimum speed following by a pausing sequence of few seconds in order to fill the Hydropore with liquid. The whole step lasts less than 1 min. After the Preload step, the bottom part of the plunger will be slightly higher than the zero graduation of the syringe (+/- 30 µl). This position will be the lower limit of the plunger movement during the remaining steps of the shearing process.

Step 2: Premix

The goal of the premix step is to increase the fluidity of the sample prior the shearing sequence by passing the sample few times through the Hydropore at a very low speed (in order to minimize the shearing of the sample at this step). After each movement of the plunger, a pausing time is applied to ensure that the totality of the sample volume passed through the Hydropore.

For samples having a concentration higher than 50 ng/µl, the Premix step is prolonged by increasing the number of up & down movements of the plunger and by applying a longer pausing time.

The duration of the Premix step is variable and depends on the concentration and the volume of the sample (higher volumes need more time to be mixed).

Step 3: Preshear

This step is only applied when the selected speed for the shearing is 59 (maximum speed). During this step, the sample is pre-sheared at an intermediate speed. This increases the efficiency and the reproducibility of the shearing for short fragments.

The preshear step increases the total duration of the process as additional up & down movements are applied.

Step 4: Shear

During the shearing step, the sample is sheared at the speed that determines its final size. The DNA is passed through the Hydropore 30 to 90 times depending on the volume and the concentration. The repeated passes through the microscopic pores at a constant speed will shear the DNA consistently and will lead to a narrow distribution of the shearing profile. More the speed is higher, more the shearing forces are higher and more the obtained fragments size is smaller and vice versa.

After each movement of the sample, a pausing time is applied in order to allow the whole sample to get through the Hydropore but also to provide the necessary time to the solution to stabilize and to the air bubbles to disappear. The pausing time is automatically calculated by the system depending on the speed, the volume and the concentration. More these parameters are bigger, more the pausing time is higher. The pausing time during the shearing phase affects the total duration of the run.

Step 5: Ending

At the end of the shearing protocol, the plunger is back to its zero position with the totality of the sample in the Hydro Tube.

Once the run is completed, a summary is displayed on the screen.

Step	Nb of up&down	Speed	Pausing time (s)
Preload	NA	1	10
Premix	**3 or 8***	**1 - 15****	**30 or 60***
Preshear	**8**	**33**	**15 or 25***
Shear	30 or 60 *** or 90 *	1 - 59	7 - 36
End	0	1	NA

**Applied if concentration is higher than 50 ng/µl*

***Premix speed automatically adjusted depending on the speed of the shearing*

****Applied if the sample volume is lower than 200 µl*

Shearing Protocols

Optimization

The Megaruptor 3 is an efficient and flexible tool to fragment DNA into long fragments from 5 to more than 100 kb with a relatively high throughput. It is flexible in term of number of samples to process (1 to 8) but also in term of sample volume and concentration. For any sample within the standard range of concentration, volume, viscosity, purity, integrity (not degraded), the shearing is expected to be reliable and reproducible whatever is the origin of the sample (species or extraction protocol). However, due to the huge variability of samples, we highly recommend to do a short optimization of the shearing parameters before processing a big number of similar samples. This will ensure more optimal and accurate shearing results.

Generally, 2 or 3 different speed settings are enough for optimization. The following section (Protocols) will help you identifying the speed settings for optimization depending on the target size and the sample volume. Our speed recommendations have been determined using the Fragment Analyzer Automated™ CE System (Agilent) combined with the High Sensitivity Large Fragment 50 kb Kit and with Pulsed Field Gel Electrophoresis (PFGE) in 1% agarose gel.

> ***NOTE**: For samples which the concentration is lower than 50 ng/µl, it is possible to use 1 to 10 ng/µl solution for the optimization step in order to not lose much of the sample.*

Protocols

Depending on the sample volume and concentration, different shearing parameters can be selected automatically by the Megaruptor 3 in order to get optimal shearing results. Thus, several protocols can be applied. As summarized in the table below, one among 3 protocols will be applied:

	PROTOCOLS		
	Standard	**Low volume**	**High concentration**
Sample Concentration	0-50 ng/µl		51-150 ng/µl
Sample Volume	100-500 µl	65 µl	200-500 µl
Fragment Length Achievable	5-100 kb	12-75 kb	5-100 kb

For conditions different from the table above, please contact Diagenode for more assistance. https://www.diagenode.com/en/pages/support

Standard Protocol

The Standard Protocol of the Megaruptor 3 covers the most common sample volumes and concentrations. It is applied when the volume of the sample is between 100 and 500 µl and the concentration is lower or equal to 50 ng/µl.

The table indicates the speed range to be selected in the software for a determined target fragment size.

Speed	Target size
1	100 - 130 kb
2	90 - 100 kb
3 - 4	80 - 90 kb
4 - 6	70 - 80 kb
6 - 11	60 - 70 kb
12 - 20	55 - 60 kb
21 - 27	50 - 55 kb
28 - 30	40 - 50 kb
31 - 32	30 - 40 kb
33 - 35	20 - 30 kb
36 - 38	15 - 20 kb
40 - 46	10 - 15 kb
49 - 56	7 - 10 kb
59	5 - 7 kb

Low Volume Protocol

Below 100 µl, the shearing may be less efficient due to a less efficient mixing of the sample. Smaller is the volume, larger is the obtained size for the same other parameters. Thus, for sample volume below 100 µl,

more optimization is required. However, a protocol for a sample volume of 65 µl has been already optimized for a perfect match with low volume PacBio® and Oxford Nanopore™ library prep.

***NOTE**:*

- *The lowest possible size with the 65 µl protocol is 10-12 kb;*
- *The shearing at volumes smaller than 100 µl can result in a slightly less reproducible shearing results.*

The table below indicates the speed range to be selected in the software for a determined target fragment size.

Speed	Target size
1	60 - 75 kb
2 - 9	50 - 60 kb
12 - 18	40 - 50 kb
21 - 29	30 - 40 kb
30 - 32	25 - 30 kb
33 - 35	20 - 25 kb
36 - 40	15 - 20 kb
46 - 59	10 - 15 kb

High Concentration Protocol

If the sample concentration is higher than 50 ng/µl, the software will automatically select the shearing parameters adapted to highly concentrated samples (which are generally more viscous). The applied parameters consist mainly on a longer premix step to reduce the viscosity of the samples and on a longer pausing time to provide enough time for the viscous samples to pass through the Hydropore after each movement.

***NOTE**:*

- *The minimum recommended volume for samples which the DNA concentration is higher than 50 ng/µl is 200 µl.*
- *When the High Concentration Protocol is applied, the total duration of the process will significantly increase comparing to the Standard Protocol.*
- *If the samples are suspected to be too viscous even if their concentration is lower than 50 ng/µl, we recommend to apply the High Concentration Protocol. Practically, enter a concentration value higher than 50 ng/µl to the software.*

For High Concentration Protocol, please refer to the speed recommendations in the table at Standard Protocol section (page 23).

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 around 5-7 kb, the Fragment Analyzer Automated™ CE System (Agilent) 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 Analyzer Automated™ CE System (Agilent) combined with the High Sensitivity Large Fragment 50 kb kit can be used.
- For fragments above 50 kb, a Pulsed Field Gel Electrophoresis instrument (PFGE) or the Femto Pulse System (Agilent) can be used.

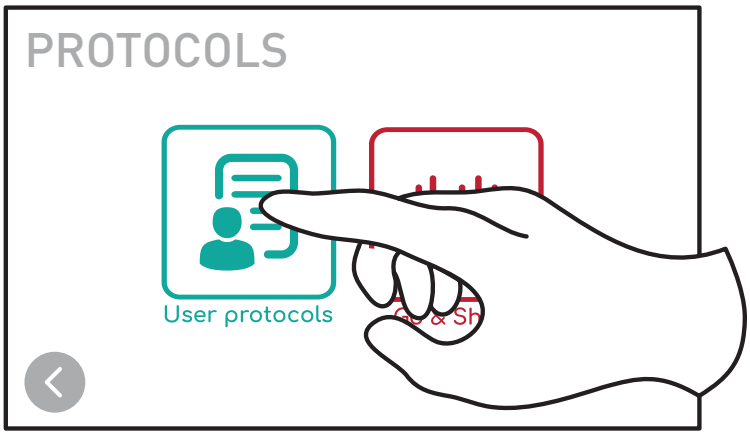
***NOTE**: When using the High Sensitivity Large Fragment 50 kb kit (Agilent), we highly recommend to use no more than 1 ng of sheared DNA for the sizing. Using more than 1 ng can result on a less consisting sizing. For the Femto Pulse, please refer to manufacturer recommendations.*

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

Additional Functionalities

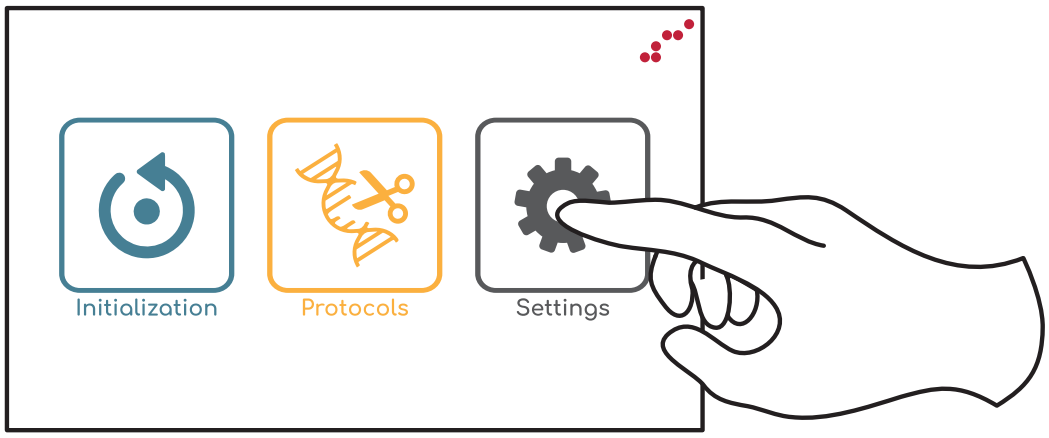
User Profiles

It's possible to save into the system up to 4 different shearing protocols/ parameters by clicking on 'User protocols' icon.



Settings

Visualize and change parameters of the Megaruptor 3 by clicking on 'Settings' icon of the top menu.



Voice

The Megaruptor 3 is a "speaking" instrument. Several messages can be displayed depending on the status of the process in order to make the device more user-friendly.

Select one of the available languages and adjust the volume of the sound. To deactivate the "speaking" option, adjust the volume at its minimum value.

Brightness Control & Screen Saver

The brightness of the screen can be adjusted using the scale.

A screen saver option is also available in the instrument in order to reduce energy consumption in case of a prolonged non-use of the instrument. This option can be selected or not. In the case it's selected, the screen will turn off after 30 min of rest. The screen saver option will not be activated during a shearing protocol. To reactivate the screen, just click anywhere on it. The previous page will then reappear.

Maintenance

The Megaruptor 3 doesn't require a specefic maintenance. However we recommend to clean the instrument and the cassette with a towel paper slightly soaked with water to remove dust and slight dirt stains. Never clean the instrument with ethanol or acetone to not damage the painting.

Always turn off the instrument after use or at the end of the day using the power button.

Troubleshooting/FAQ

The volume of DNA sample appears to be smaller after processing.

It's normal that 5-15 µl of the sample can be lost due to pipetting errors, plastic retention and dead space inside the consumable. We highly recommend to shear your desired final volume + 15 µl extra. In all cases, the real sample volume should be entered to the volume settings page (ex: for 200 µl final volume, prepare 215 µl sample and enter 215 µl as the sample volume). If the sample loss is higher than 15 µl, shortly spin the Hydro Tube and measure again the volume. If this does not bring an answer, please contact Diagenode (https://www.diagenode.com/en/pages/support).

The shearing profile shows more than one Peak after processing.

Check if the original sample (not sheared sample) is not already degraded. If it's not the case, check if the sample has splashed up onto the walls of the Hydro Tube. Always, spin the Hydro Tubes before processing the samples. Also, check if the real sample volume has been entered to the software; a non-correct volume settings will affect the shearing efficiency and reproducibility.

A big air bubble is visible in the syringe on top of the sample during the shearing.

This is totally normal as the movement of the sample inside the syringe is carried out via an air cushion.

A big and consistent air bubble is visible in the syringe on the bottom of the sample during the shearing.

Check if the correct volume has been entered to the software, if the entered volume is bigger than the real volume, an air bubble will be created in the bottom of the syringe. Also, spin the samples before processing.

During the Preload and/or Premix step, the sample is not fully drawn into the syringe.

The speed of the Preload and the Premix steps and the pausing time after each movement of the plunger are optimized to avoid such kind of issue. However, in case of too viscous samples, this is a normal observation mainly in the Preload or Premix steps. After few passes, the samples should become more fluid and should be gradually drawn into the syringe. Also, check if the entered volume is consistent with the real sample volume.

The sample does not pass at all through the hydropores even after several movements of the plunger.

This can be due to very viscous samples, a consequence of extremely high molecular weight DNA or of impurities present in the sample. Refer to the Sample Preparation section (page 10).

The DNA fragments are not at the expected size.

The provided protocols will help you to optimize the shearing parameters to your samples. If the sample is pure and not degraded, it is expected to be sheared at a size close to what is indicated in the protocol. If the obtained size is obviously different from the expected size range, this can be due to several reasons:

- Issue with the sizing instrument: some instruments are not suitable for large fragments or are simply not working properly. Repeat the analysis with a control sample (ex: intact lambda DNA);
- Sample is already degraded before shearing. Always analyse in parallel sheared and non-sheared samples;
- Wrong speed parameters were entered to the software. After setting the shearing parameters, the software displays a summary of entered parameters. Check carefully the entered parameters before starting the shearing. It's also possible to check the shearing parameters during an ongoing run via the information button. In addition, the software keeps in memory the settings of the last run (even when switching off the instrument). It is still possible to check these parameters even after the end of the run.

The shearing results of samples from the same run are not reproducible.

As long as the samples are pure, intact and having the same volume with similar aspect and concentration, the shearing results should be reproducible (CV ‹ 15 %). If it's not the case:

- Check the entered shearing parameters;
- Check if all the samples have the same volume and concentration range;
- Use preferably samples from similar origins / species / extraction protocols in a same run;
- Check if the hydropore-syringes assembly is well tight prior to installing them on the cassette.

How many times can we use the consumables even for the same samples?

The sterile assemblies Hydropore-Syringe, and the Hrydro Tubes are intended for single use. In case of high volume of sample, please, contact Diagenode (https://www.diagenode.com/en/pages/support).