HOLOGIC®
Diagenode

Megaruptor® 3 DNA QC Kit

Track the efficiency of your Megaruptor® 3

V1 10_2025 Revision 001

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Dear customer,

This DNA Quality Control Kit has been specifically designed to help you monitor the performance of your Megaruptor® 3 and determine when servicing may be necessary. In case your QC test results do not meet our quality standards, please contact Hologic Diagenode for guidance and re-commendations. On the other hand, if your QC test passes, we wish you continued success with your Megaruptor® 3!

Thank you for your confidence in Hologic Diagenode.

Best regards,
The Hologic Diagenode Customer Support Team.

PRINCIPLE

The Hologic Diagenode Megaruptor® 3 DNA QC kit is used to evaluate the DNA shearing performance and consistency of your Megaruptor® 3 or Megaruptor® 3 HT under specific conditions.

It involves shearing eight unsheared DNA samples with known concentration and volume and comparing the resulting fragmented DNA profiles to a Control DNA sample, pre-sheared under identical conditions by Hologic Diagenode. Quality and uniformity of the shearing process are assessed by calculating the coefficient of variation (%CV) of the fragment sizes for the eight DNA samples and comparing it to the Control sheared DNA.

This ensures that your Megaruptor® 3 performs within the intended specifications.

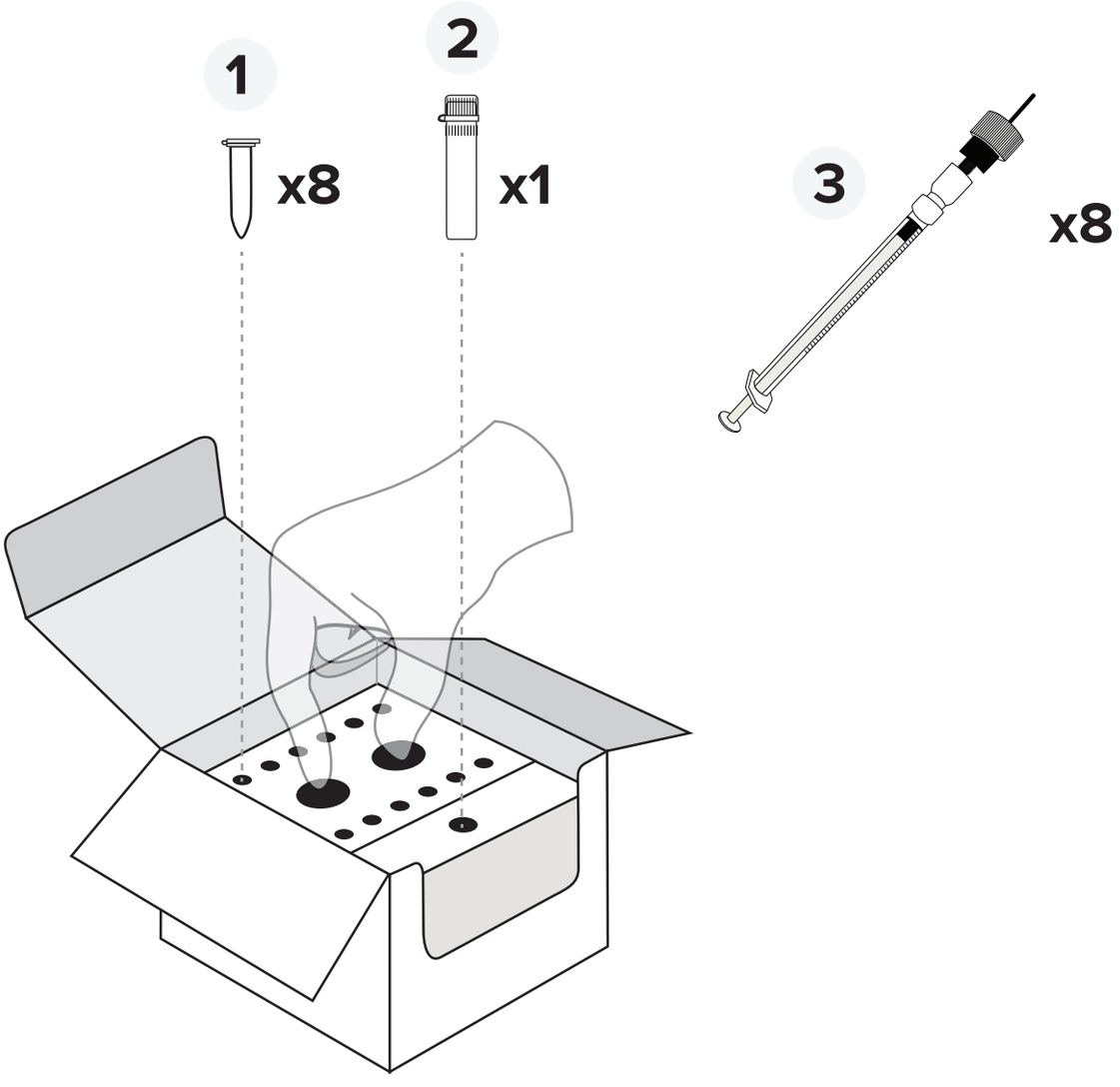
KIT CONTENT

The kit contains sufficient reagents for performing one shearing experiment.

Description	Quantity	Storage
Unsheared DNA (10 ng/µl)	8 tubes (200 µl/tube)	4 °C
Control sheared DNA 10-15 kb (10 ng/µl)	1 tube (10 µl/tube)	4 °C
Megaruptor® 3 shearing consumables	8 consumables	RT
Datasheet of the control	1	-

Expiry Date: 1 year from the date of receipt

1. Unsheared DNA: high molecular weight gDNA (10 ng/µl) in TE buffer in Hydro Tubes
2. Control sheared DNA: gDNA sheared to 10-15 kb (10 ng/µl) in TE buffer
3. Megaruptor® 3 shearing consumables



REQUIRED MATERIALS NOT PROVIDED

- Megaruptor® 3 or Megaruptor® 3 HT (Cat. No. B06010003)
- Femto Pulse System and Genomic DNA 165 kb Kit (Agilent)

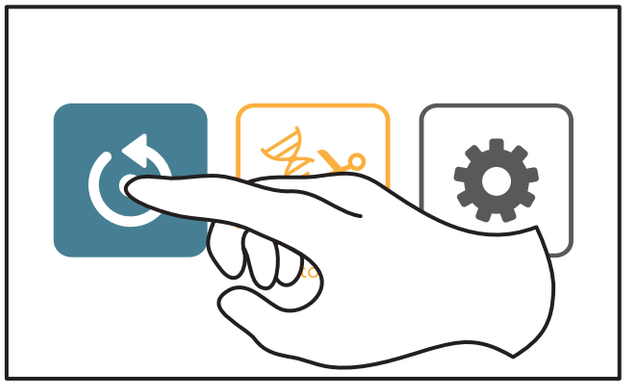
  or

- Fragment Analyzer system and HS Large Fragment 50 kb Kit (Agilent)

1. Switch ON the Megaruptor® 3.

2. If the Megaruptor® 3 was not initialized during startup the user will be invited to initialize the system.

   Select the blinking “Initialization” icon in the top menu.

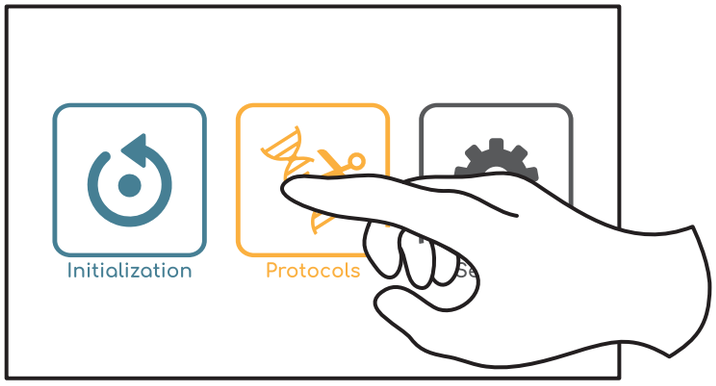


If the cassette is already installed on the base unit, as indicated on the screen, remove the cassette by shifting it up and wait 3 seconds. A brief movement (down then up) of the 2 metallic arms will set the device at its zero position.

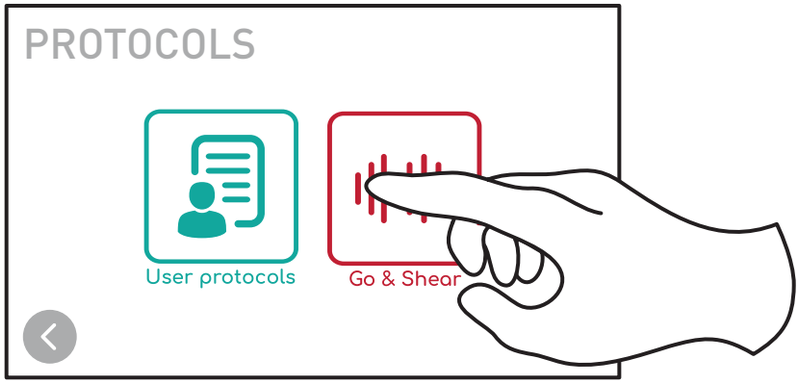
If the cassette is not yet installed on the base unit, the initialization step will be done 3 seconds after clicking on the initialization icon.

3. Set the shearing parameters:

- Select the “Protocols” icon in the top menu.



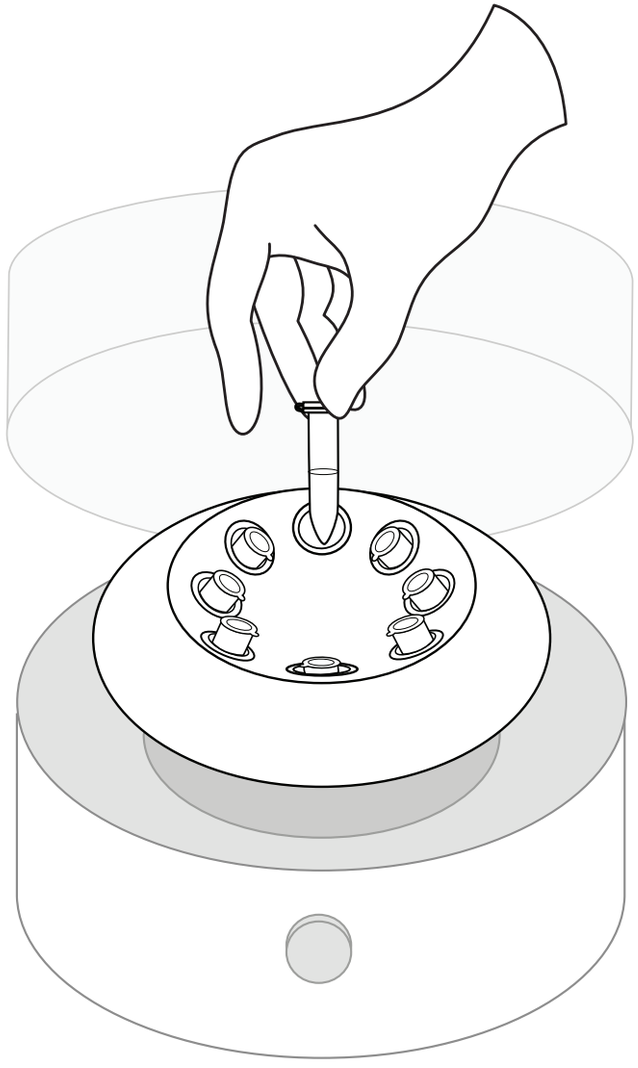
- Select the “Go & Shear”



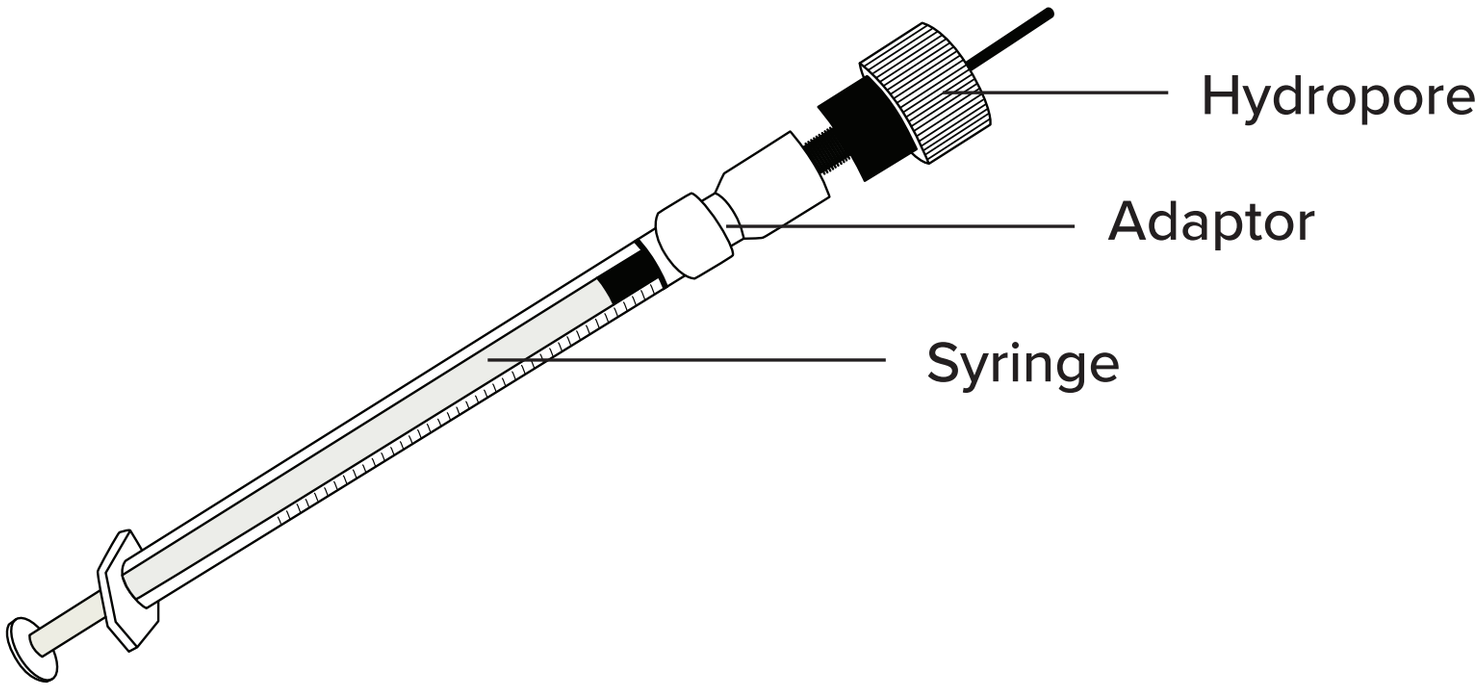
- Enter the following parameters:

  Volume = 200 µl
  Concentration = 10 ng/µl
  Speed = 40

4. Centrifuge the 8 Unsheared DNA tubes for ~10 seconds and bring to room temperature for 15 minutes before use.



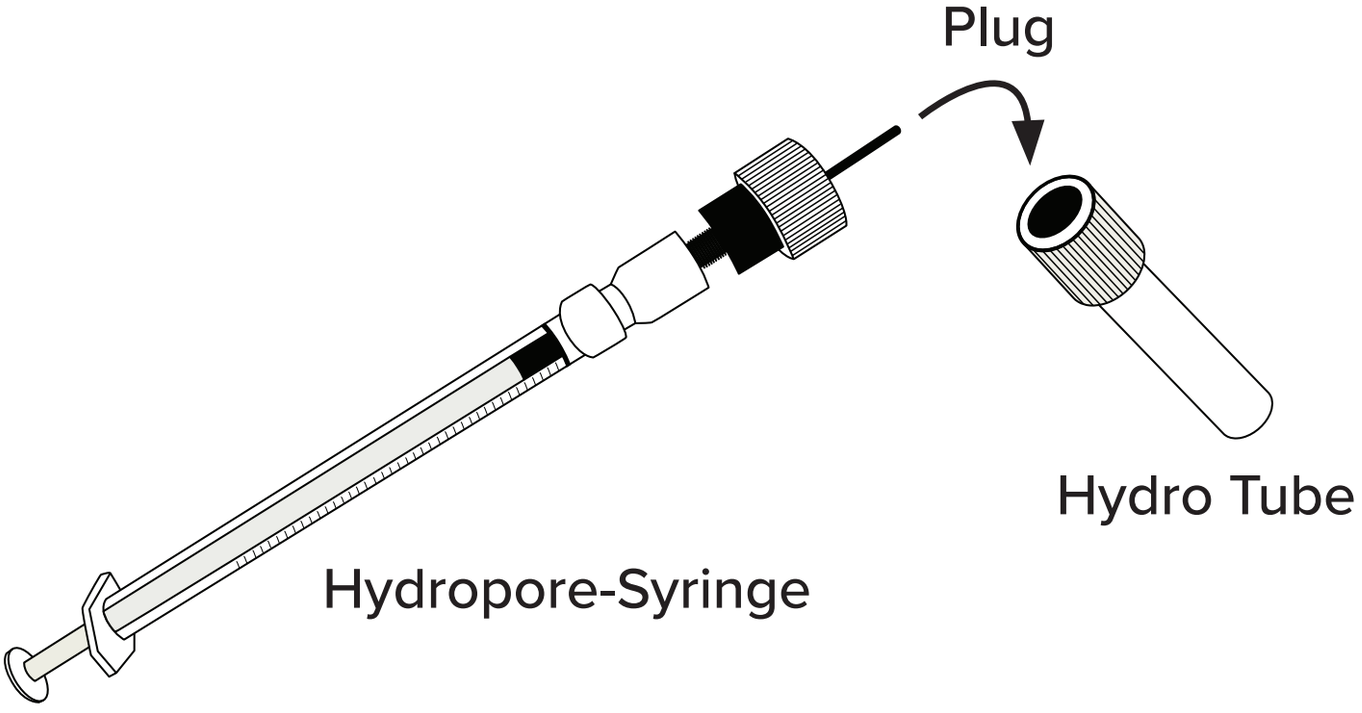
5. Unpack all 8 Megaruptor® 3 shearing consumables and check if they are correctly tightened.



Watch the video
on our Youtube channel

How to tighten Megaruptor shearing kit correctly

6. Plug the 8 Unsheared DNA **in Hydro Tubes** into the 8 Megaruptor® 3 consumables. There is no screwing needed but make sure the Hydro Tube is well inserted into the Hydropore.



7. Immediately install all 8 Megaruptor® 3 and Unsheared DNA in Hydro Tubes assemblies into the Megaruptor® 3 8 sample cassette as explained in the Megaruptor® 3 manual.

Note: *The 8 sample cassette should be full with consumables and samples for a valid QC.*

8. Close the cassette and launch the Megaruptor® 3 run using the parameters entered in step 3.

Watch the video
on our Youtube channel

How to set up your samples in the Megaruptor® 3

1. Analyze the sheared samples and the control sheared DNA sample (optional) on

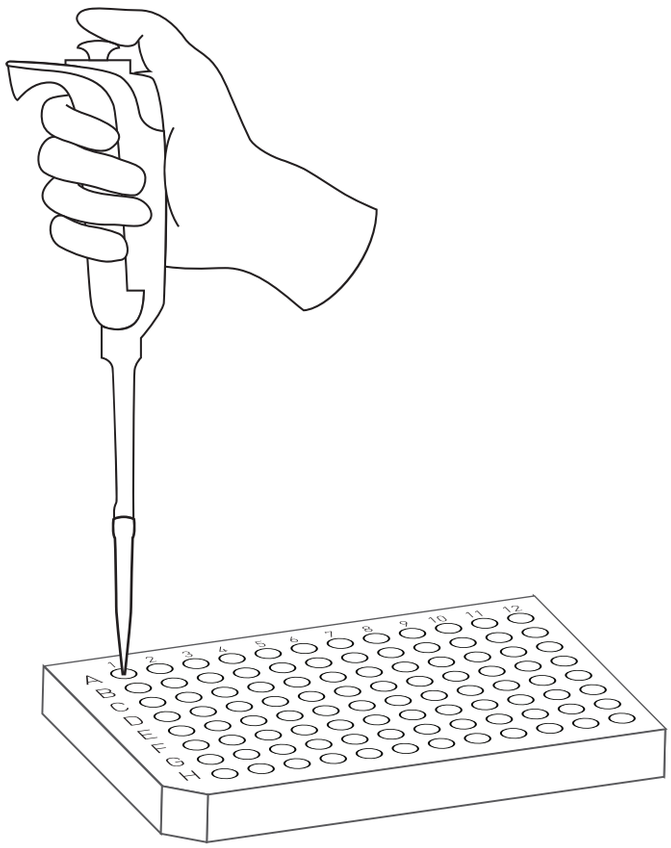
- an Agilent Femto Pulse system and a Genomic DNA 165 kb Kit: go to section **9a**

- an Agilent Fragment Analyzer system and HS Large Fragment 50 kb Kit: go to section **9b**

Note: *Agarose gels or Pulse Field Gel Electrophoresis systems are not recommended as they do not provide sufficient resolution to quantitatively assess the Megaruptor® 3 DNA QC results*

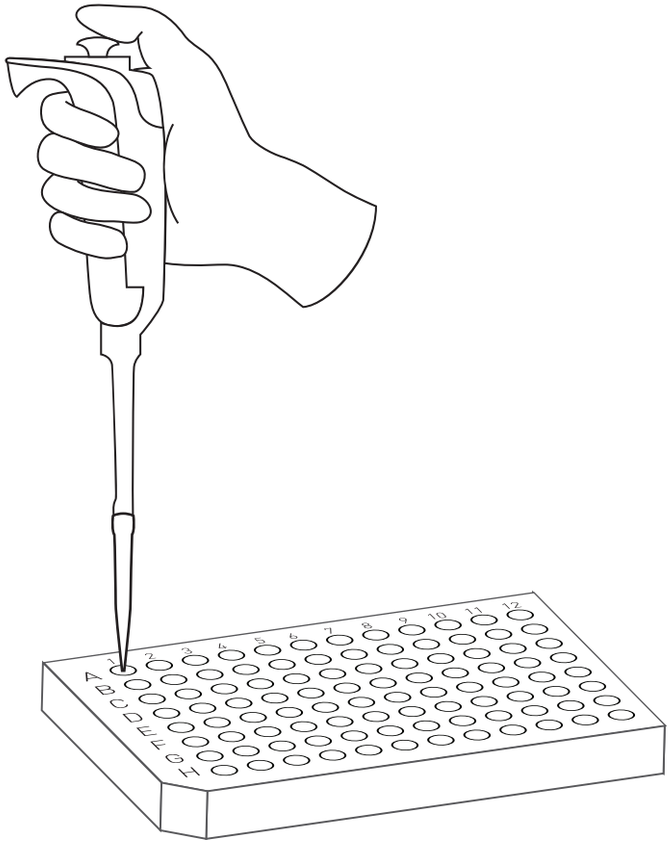
1a. Using an Agilent Femto Pulse system and a Genomic DNA 165 kb Kit

- Dilute each sample to 200 pg/µl in TE buffer, e.g. dilute 2 µl sample with 98 µl TE 1x or TE 0.25x.
- Run 2 µl of each diluted sample on the Femto Pulse system according to the manufacturer's recommendations.



1b. Using an Agilent Fragment Analyzer system and HS Large Fragment 50 kb Kit

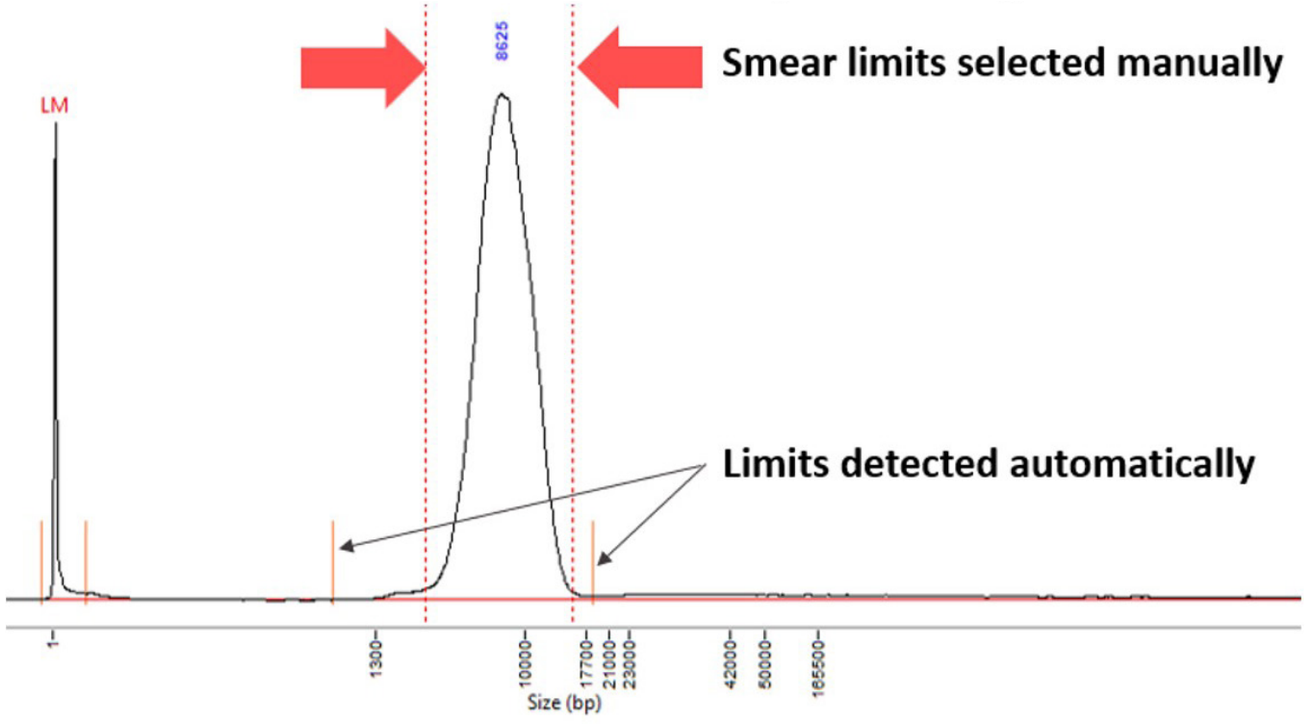
- Dilute each sample to 500 pg/µl in TE buffer, e.g. dilute 5 µl sample in 95 µl TE 1x or TE 0.25x.
- Run 2 µl of each diluted sample on the Fragment Analyzer system according to the manufacturer's recommendations.



To determine the average size of the smear, it is necessary to manually define the smear limits instead of referring to the limits that are automatically detected by the software (see figure below).

Record the average size for each sample smear using smear analysis option available on the Agilent ProSize software.

Note: *Do not use the Peak size but only the Average size.*



2. Use the recorded Average size of the Smear of all 8 samples for the calculation of:

- mean size (μ)
- standard deviation (σ)
- coefficient of variation (%CV = σ/|μ|*100%)

Shearing results are considered as excellent or very good if

μ = 10 to 15 kb and CV% < 15%

You can find an .xls template to facilitate calculations at

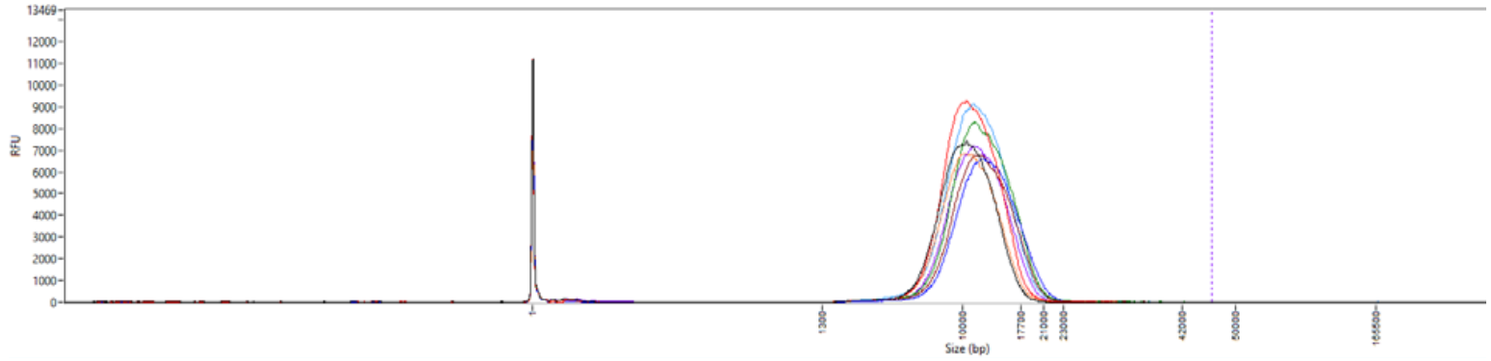
Your QC failed or you need help to perform the QC?

Please contact us at:
www.diagenode.com/en/pages/contact

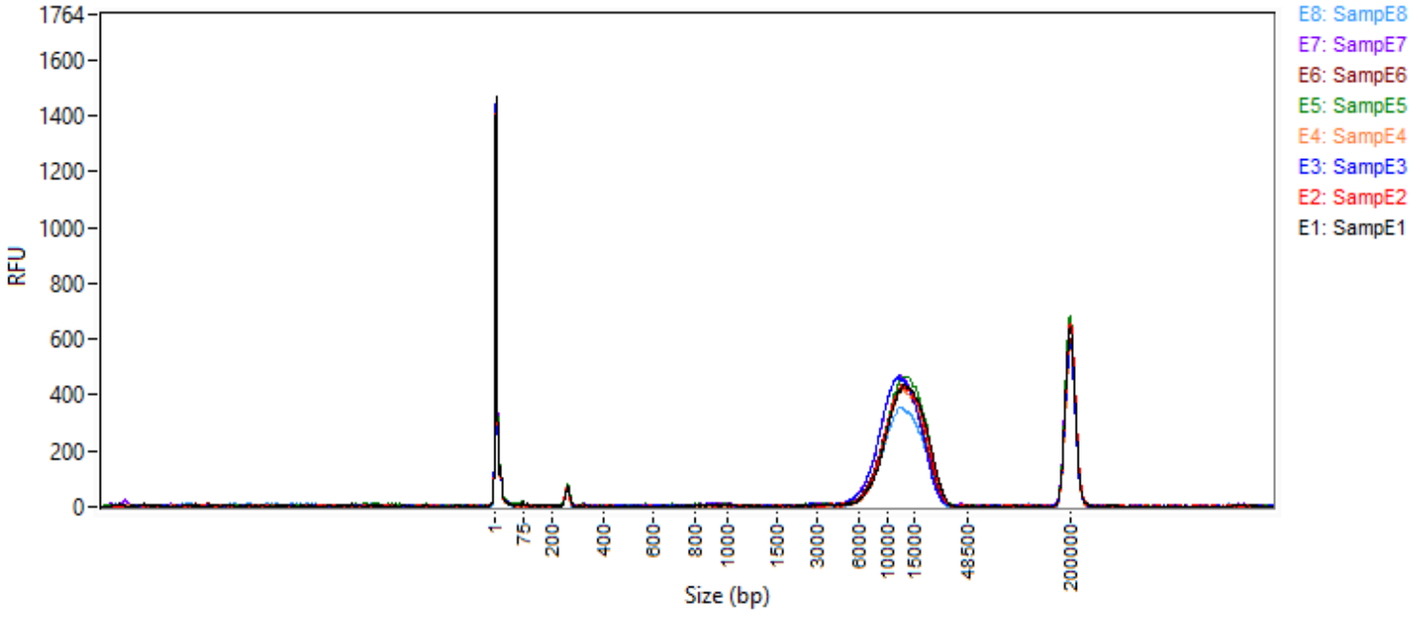
TECHNICAL DATASHEET

Human genomic DNA was sheared using the Megaruptor. After shearing, the DNA was analyzed using 2 different methods:

1. Agilent Femto Pulse system and a Genomic DNA 165 kb Kit



2. Agilent Fragment Analyzer system and HS Large Fragment 50 kb Kit



Test method	Expected results (%CV ≤ 15%)	Results
Femto Plus	Mean size between 10 and 15 kb	Passed
Fragment Analyzer	Mean size between 10 and 15 kb (NB: the sizing accuracy may be slightly lower than the Femto Pulse)	Passed

This product is in accordance with the expected specifications.

Jan Hendricks

Kit & Antibody Production

Hologic Diagenode

June 5, 2025

www.diagenode.com

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