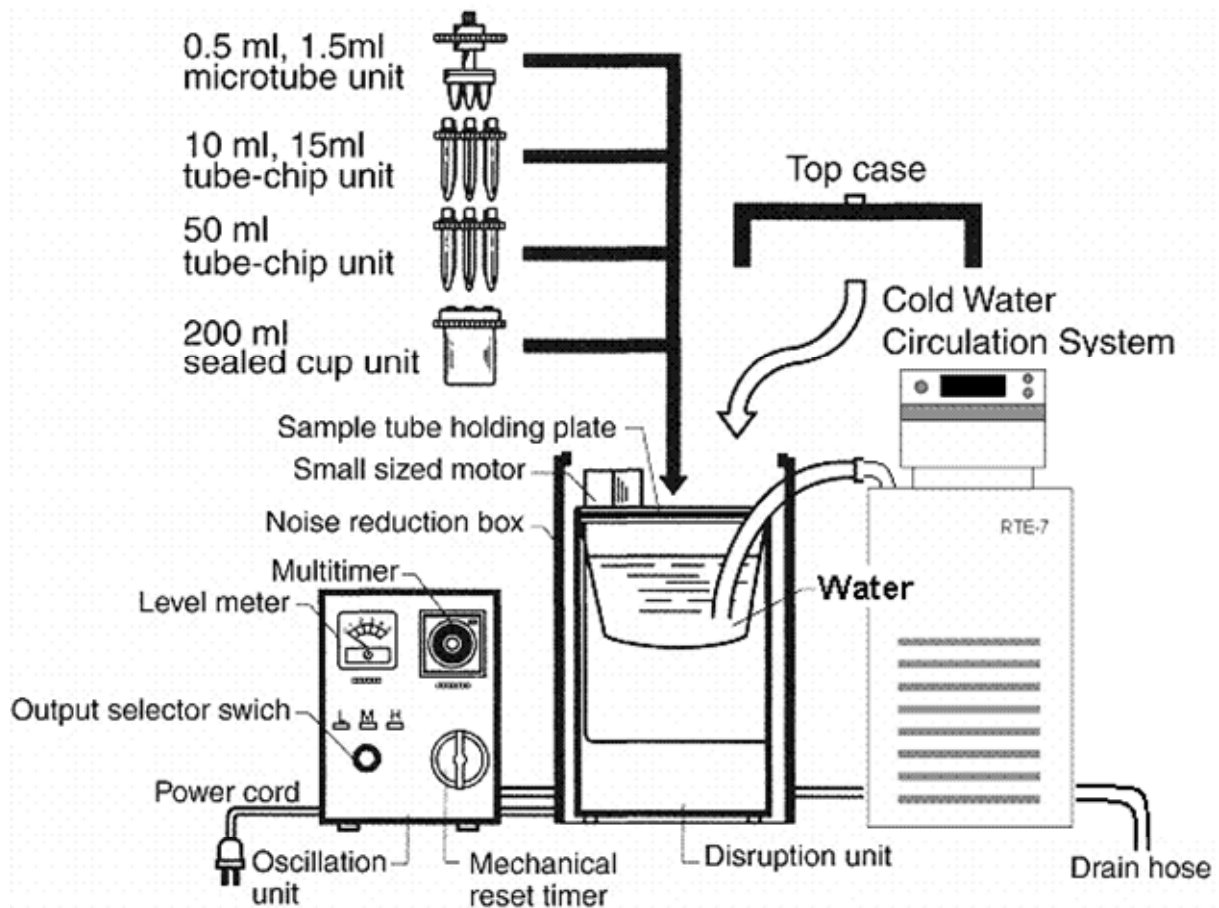


# Bioruptor User manual

Diagenode sa  
Allée du 6 août, Bât B6  
Sart Tilman · 4000 Liège  
BELGIUM



The complete Bioruptor system with the different adaptor units and the optional cold water circulation system.

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# 1 Introduction

## ***What is the difference between the Bioruptor and a traditional probe system?***

Traditional sonicators utilize a probe directly in contact with the biological sample. This has major drawbacks in terms of reproducibility as the sonication energy depends on the depth of the sonication probe in the liquid. Moreover the probe system is tedious to work with, produces foam, and only one sample can be treated at a time. Also contamination between different samples is frequently experienced. Additionally, the probe system generates aerosols, which are hazardous by biosafety rules.

The **Bioruptor** System is based on a water bath with high power ultrasound generating elements located below the tank.

With the **Bioruptor**, 6 to 12 closed tubes can be sonicated together and the continuous rotation of tubes allows even distribution of the energy.

With a better control on the parameters, the **Bioruptor** enables the automation of the sonication step which guarantees higher reproducibility and constant results.

The frequency of the ultrasound energy produced by the **Bioruptor** and a probe sonicator is equivalent (20 kHz).

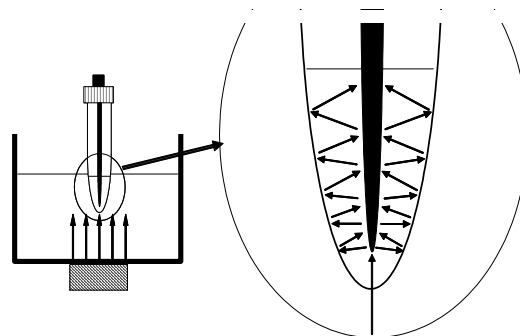
## ***What is the effect of ultrasound on biological samples?***

A generally accepted view is that ultrasound produces a gaseous cavitation in the liquid. This term describes the formation of small bubbles from dissolved gases or vapors due to alteration of pressure. These bubbles are capable of resonance vibration and produce vigorous eddying or microstreaming, which is sufficient to break cells. Also, the fragmentation of DNA takes place as a consequence of mechanical stress or shear from the bubbles.

With a probe sonicator, the microstreaming phenomenon is limited to the vicinity of the probe, whereas for the **Bioruptor**, the whole volume of water present in the tank is exposed to ultrasound energy.

For 15 ml or 50 ml tubes, a metallic bar in contact with the sample facilitates the transfer of the ultrasound inside the tubes. This metallic bar is not a probe but “reflects” the ultrasound originated from the water bath and improves the sample sonication efficiency by a patented resonance system. Produced in stainless steel it is not prone to corrosion.

The figure below schematizes the resonance of the ultrasound on the metallic bar.



The following references are useful to better understand the sonication process:

- Elsner, H., Lindblad E. Ultrasonic degradation of DNA . DNA, **8**, p697-701 (1989).  
Hughes D., Nyborg W. Cell disruption by ultrasound. Science, **138**, p108-14 (1962)

## **2 Getting started with the Bioruptor**

To achieve good reproducibility with the Bioruptor, it is important to read entirely this user manual to get familiar with all of the Bioruptor's components.

### **2.1. Water bath**

#### **2.1.1. Level of water**

The transfer of the ultrasounds from the generators located below the tank to the samples is done through a water bath.

The level of the water has been optimized and should always reach the blue line indicated on the inner wall of the tank (Fig 1)

Tap water or distilled water can be used to fill the tank.

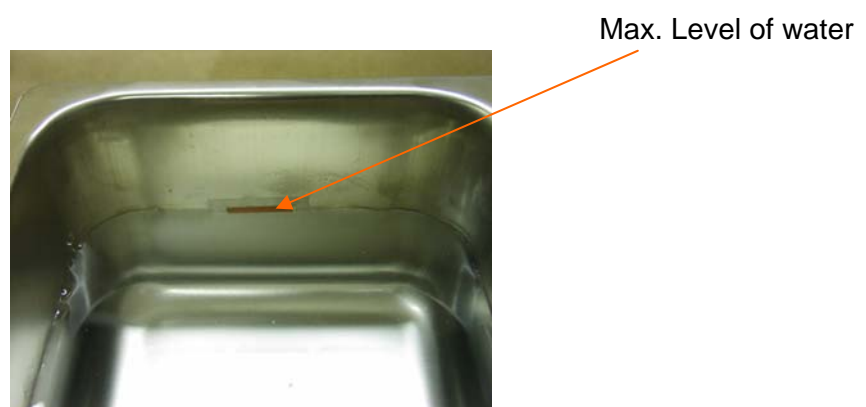


Fig. 1

#### **2.1.2. Water temperature**

Propagation of ultrasound in a liquid unavoidably produces heat.

To ensure the best preservation of the sample, it is necessary to start the sonication process with cold water in the water bath.

This can be obtained either by manual or automatic temperature control

##### **Manual temperature control**

A "pre-cooling" of the Bioruptor's tank with crushed ice 15 min. before starting the first round of sonication is recommended to avoid water heating too quickly due to thermal inertia (the tank and the ultrasound generating elements are generally stored at room temperature).

Fill the water bath to the indicated level with cold water. A good practice is to keep a stock of water at 4°C. For the cold condition to last longer in the tank during sonication, it is possible to supplement the water with floating layer of crushed ice (Max. 0.5 cm) but the total level should not exceed the blue line.

At the end of a typical sonication time (10 min., 30sec "on", 30 sec "off"), the temperature in the water bath should remain below 10°C.

Note: The permanent installation of the Bioruptor in a cold room is possible, although not sufficient to avoid the temperature increase due to sonication. This location would only replace the "pre-cooling" step described above.

### Automatic temperature control (Optional)

A refrigerated circulation bath can be used to guarantee the automatic temperature control of the water bath during the whole sonication process.

The optional circulation bath RTE-7 features two pumps (“IN” and “OUT”) and produces a regular water flow with a constant water level in the tank. (Fig 2)

An additional regulating valve is adapted on the water circuit going from the refrigerating unit to the Bioruptor. In this way, the water flow can be reduced to an optimal level.

Keep the water flow tiny to not interfere with the resonance process in the water bath (Flow around 500 ml/ minute).

This instrument can be ordered directly through Diagenode with all the required tubing (Cat #RTE-7D1).



Fig.2

### 2.2. Motorized lid

The lid ensures the optimal position of the different sized tubes in the water bath during sonication.

The blue row is always easily placed into its location (Fig 3).

The motor (Fig 3 - arrow) keeps all the samples in constant rotation.

Note:

Avoid immersion of the motor into water.

When in motion, do not impede the rotation of the blue row.



Fig 3

### 2.3. Tube Holders

Several sizes of tubes can be used with the Bioruptor.

The maximum and minimum sample volumes to be used with each container are given in the table below.

0.5 ml micro- tube	Less than 100 $\mu$ l of solution per tube Minimal sonication volume 10 $\mu$ l
1.5 ml micro- tube	Less than 300 $\mu$ l of solution per tube Minimal sonication volume 100 $\mu$ l
15 ml tubes	Less than 2 ml of solution per tube Minimal sonication volume 500 $\mu$ l
50 ml tubes (Falcon or Corning)	Less than 20 ml of solution per tube Minimal sonication volume 3 ml
50 ml tubes (Nalgene)	Less than 8 ml of solution per tube Minimal sonication volume 1 ml
200ml cup	Less than 50 ml of solution per cup Minimal sonication volume 10 ml

Micro tubes are simply closed and installed in the rotor.

For the sonication of larger volumes (10, 15, 50 ml tubes and the 200 cup), a stopper with a metallic bar has to be used for a better resonance efficiency. The metallic bar does not vibrate but “reflects” the ultrasounds originated from the bottom of the tank and initiates a resonance process (see 1 Introduction).

### 2.3.1. 0.5ml micro tube unit (MAT-05)

Turn the microtube holder of the lower part to a counter clockwise, and take it off.

Place microtubes in the unit and attach again the lower part to the upper one.

If the holder is not completely filled, the microtubes have to be balanced opposite to each other (like in a centrifuge rotor).

Thin-walled 0.5 ml tubes specially adapted for thermal cyclers could be used for sonication but all new material has to be tested with water-filled tubes in a typical sonication cycle. Once selected, stick to a particular brand of tubes for consistent results.



Fig 4

### 2.3.2. 1.5ml micro tube unit (MAT-15)



Fig 5

See above. For consistent results, always use the same brand of Eppendorf tubes.

2ml Eppendorf tubes should not be used with the Bioruptor.

All Eppendorf tubes are generally in polypropylene. Special Eppendorf tubes in TPX plastic with a slightly better ultrasound transfer rate are available from Diagenode (Cat # M-50001).

### 2.3.3. 10 an 15 ml tube units (Fig 7 an 9)

For these tubes, the sample holding plate is the NG-6 (Fig 6). This gear plate can accommodate up to 6 tubes.

If the holding plate is not completely filled, the tubes have to be balanced opposite to each other (like in a centrifuge rotor). A single tube can be put in central position.

The complete resonance chip (including O ring) can be sterilized by autoclaving. After more than 20 autoclave cycles, the O ring (Fig 8) should be replaced (Cat # P-10).



Fig 6

#### 10 ml tubes (Fig 7)



Fig 7

O ring for the  
10ml chip unit



Fig 8

#### 15 ml tubes (Fig 9)



Fig 9

The adaptors for 15 ml tubes are available in two dedicated models: blue (Falcon tubes) and orange (Corning tubes). If you use another brand of tube, please try the one which fits the best (the metallic bar should not touch the wall of the tube).

By removing the black knob (Fig 10), it is possible to replace the O-ring. (Cat # P-10A)



Fig 10

**Notes:**

- **Quality of the 15 ml tube** (Falcon or Corning): “Hard” plastic (Polystyrene or polyethylene-Crystal clear tube) can be used as well as “soft” plastic (Polypropylene, more white aspect), but you should stick to one kind as transfer of ultrasonic waves is different (Hard plastic is more efficient)

- When using the 15 ml tubes, do not forget to insert the **aluminium ring** (Fig 10) to ensure an optimal position of the tube during sonication.

**2.3.4. 50 ml tube units**

Three different models of adaptors for 50 ml tubes are available. For these tubes, the sample holding plate is the NG-50-3 (Fig 11). This gear plate can accommodate up to 3 tubes. A central position is available if only one tube is sonicated.



Fig 11

**Nalgene type:** (Chip 50)



Fig 12

The Nalgene tube in polycarbonate has a conical shape which is well adapted to the use in the Bioruptor. These tubes (Fig 12), although more expensive than the classical 50 ml tube (Falcon or Corning), offer the best efficiency in term of transfer of the ultrasound energy. These tubes can be ordered directly from Diagenode (Cat # 3105-0050).

By removing the black knob (Fig 13), it is possible to replace the O-ring (Cat # P-22A).



Fig 13

**Falcon or Corning Type: FT-50WS or CT-50WS**



The adaptors for 50 ml tubes are available in two dedicated models: Blue (Falcon tubes) and orange (Corning tubes) (Fig 14). If you use another brand of tubes, please try the one which fits best (The metallic bar should not touch the wall of the tube).

Fig 14

By removing the black knob (Fig 15), it is possible to replace the O-ring (Cat # P-22B).

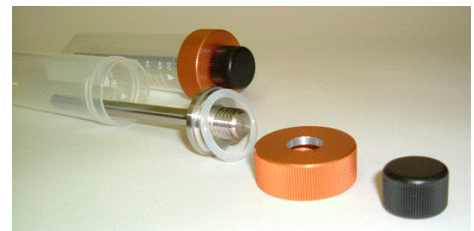


Fig 15

**Note:**

The quality of the 50 ml Corning tube: “hard” plastic (polyethylene-ref Corning 430304 ) can be used as well as “soft” plastic (Polypropylene, ref. Corning 430290 ) but you should stick to one kind as transfer of ultrasonic waves is different (Hard plastic is more efficient). For Falcon tubes, only polypropylene tubes are available.

**2.3.5. 200 ml Cup**

This cup (Fig 16) is dedicated to the mild sonication of sample up to 50 ml. The presence of a flat ring on the stopper (Cat #. P-62N) ensures the absence of aerosols during all the sonication process.



Fig 16



## 2.4. Control Unit



Fig 17

The control unit allows the automatic production of On / Off cycles to preserve the samples from rapid heating due to the ultrasound energy. This is achieved by the Multitimer located on the upper right part of the control unit (Fig 17).

The setting of the “Off” time is done by the green needle which can be handled by turning the external surface of the dial.

The setting of the “On” time is done by the red needle which can be handled by turning the internal knob of the dial.

The unit is the minute.

The Bioruptor is active as soon as you have turned the mechanical timer (Fig 18) of the control unit clockwise and set the time (Max. time: 15 Min.).

The time set is the total time which is the sum of the “On” and “Off” cycles.

Alternatively, turning the timer knob counter clockwise will set the Bioruptor on a permanent position (always cycles “On” and “Off”).

To stop the process, manually set the timer knob on vertical off position.

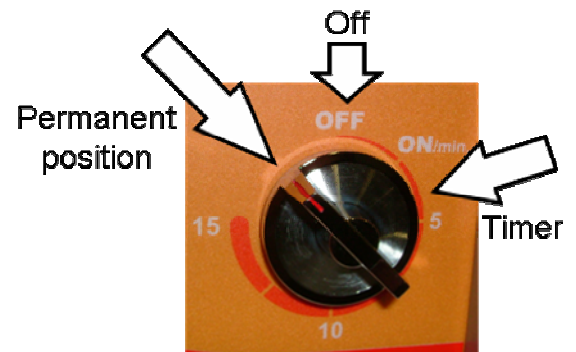


Fig 18



Fig 19

Output selectors switch (Fig 19):

There are three power settings for the sonication process. These can be automatically selected by the power knob:

L:	low	130 W
M:	Medium	160 W
H:	High	200 W

A meter is also present on the control unit to visualize the sonication intensity.

### **3 Optimization of sonication parameters with the Bioruptor**

The Bioruptor being a research instrument allows the user to select the different parameters of sonication. Once optimal conditions are determined for a particular biological sample (fixed sample volume and density), the Bioruptor will ensure an excellent reproducibility of the whole process.

The determination of the optimal parameters is done empirically by doing preliminary tests with samples of different density in the different tubes and by analyzing the results.

#### **3.1. Starting points:**

The starting sonication parameters shown below should give expected results for a maximum range of applications.

**Water level in the tank:** Precisely to the blue line

**Temperature:** Melting ice temperature (see section 2.1.2. for details)

**Power:** set the Output selector switch on High (H)

**Cycling parameters:** Set the Multitimer on 30 sec. "On", 30 sec. "Off"  
(Red and green needles set on 0.5 Min.)

<b>Total time:</b>	- PCR tubes:	6 min.
	- Eppendorf tube	8 min.
<u>Select</u>	- 15 ml tubes (Polypropylene)	12 min.
	- 50 ml tubes	15 min.

<b>Sample volume:</b>	- PCR tubes:	100 µl
	- Eppendorf tube	300 µl
	- 15 ml tubes (Polypropylene)	2 ml
<u>Select</u>	- 50 ml tubes (Nalgene)	8ml
	- 50 ml tubes (Falcon or Corning)	20 ml

### 3.2. Optimization test:

Obtaining constant sonication results with biological material implies a strict quality control of the sonicated samples.

Biological materials such as cell lyses extract, DNA solution or liposome suspension are a very complex mixture of molecules. The ability to disrupt by sonication one of the molecules in the mixture depends not only on the individual molecule concentration, but also on the eventual presence of contaminants.

The viscosity of the solution is another factor that can affect the sonication efficiency, as the cavitation process (see introduction) is dependent of the viscosity coefficient of the solution.

Additionally, for ChIP assay users, the fixation step (percentage of formaldehyde, duration and temperature) is a key factor that can affect the sonication results due to protein cross-linking.

The best way to start optimization tests with the Bioruptor is to collect a large quantity of the biological material to be sonicated. This material should be characterized enough to be produced easily and available in a sufficient quantity to repeat the optimization tests.

For example, a nuclear extract should be always produced with the same protocol and generated with a fixed number of cells.

As the sample concentration is a key factor to calibrate the sonication efficiency, several aliquots of the starting material should be produced by dilution with the sonication buffer.

For example 6 aliquots of the starting material could be designed as follow: 1X, 2X, 4X, 6X, 8X, 10X.

The aliquot volume in each tube should be fixed and should correspond to the tube chosen for sonication (see table in section 3.1).

Sonicate the 6 tubes together using the sonication parameters listed on the starting points (3.1) and analyze the results.

**If results show not enough disruption**, dilute again the samples stepwise. There is apparently a threshold of sample concentration above which a complete disruption does not occur. If too concentrated, the sonication time could be increased without ever obtaining a complete disruption. This seems to be due to viscosity parameters of the sample which hamper the cavitation process (see introduction).

**If too disrupted**, either concentrate sample or reduce the total time of sonication.

A **fine-tuning** of the On / Off cycles and of the other sonication parameters can be done once a satisfactory result has been obtained on a specific sample concentration.

The optimal conditions should give excellent reproducibility in term of sonication results (i.e. fragment size) and preservation of the sonicated material (no overheating).

#### **Important note when testing different sonication vessels:**

15 and 50 ml tubes are sonicated in the Bioruptor with a metallic bar which triggers the resonance effect and improves the sonication efficiency (see introduction). This implies that, for a same sonication result, one could select a higher sample concentration in 15ml tubes compared to a sonication done in smaller containers (Eppendorf or PCR tubes) which are sonicated without resonance effect. In other words, if you optimized the sonication parameters in 15 ml tubes, the same sample concentration wont necessary be adapted to the sonication in Eppendorf tubes and higher sample dilutions for the Eppendorf tubes should be tested.

## **4 Suggested Sonication protocol for Molecular Biology**

### **4.1 ChIP Assay - DNA fragmentation with the BIORUPTOR**

#### **4.1.1. Protocol to obtain up to 500 bp DNA fragments from Drosophila embryos or cell lines**

- For the use in Falcon tubes, Wet weight of biological material (Cell or nuclei pellets, embryos etc.) should not be more than 1 g. in 10 ml buffer. Higher concentrations will lead to partially fragmented DNA.
- For the use in Eppendorf tubes, a lot of customers have reported best results with a max. Concentration of one million cells (HeLa, U2OS or 293 cells) in 300µl sonication buffer.

For sonication, any buffer is suitable, depending on special requirements. A good practice is to use the buffer and extraction procedure already in use with a previous probe sonicator. If you do not have established extraction protocol, refer to section 4.3.6.

#### **Sonication condition:**

- **Bioruptor always on “High”.**
- **Sonication in 1, 5 ml Eppendorf tubes** filled with max.300µl sonication mixture.

Fill the tank with cold water (4°C), supplement with 0,5 cm crushed ice.  
Sonicate for 6- 8 min total time. (30 sec.”on”, 30 sec. “Off”)

- **Sonication in 15 ml Falcon tubes** filled with Max. 2 ml sonication mixture.

Fill the tank with cold water (4°C), supplement with 0,5 cm crushed ice.  
Sonicate for 10-12 min total time. (30 sec.”on”, 30 sec. “Off”)

- Reverse cross link the sample and analyze on gel electrophoresis.

#### **Note:**

- If a high molecular weight DNA smear is observed instead of a high proportion of 500 bp DNA fragments, the amount of starting material (Cells or Embryos) is too high and it has to be lowered in the sonication buffer.

- This protocol is a starting point and the total time of sonication can be lengthen and/or “On”-“Off” cycling parameters can be adapted to special requirements. For example, yeast cells might require a bit longer sonication times.

#### **4.1.2. Protocol to obtain 350-200 bp DNA fragments from Drosophila embryos or cell lines**

- Starting material should be fixed a bit longer than usual to protect proteins from high energy ultrasonic disruption. This could contribute to the preservation of epitopes for the subsequent immuno-precipitation step.
- **Sonication in 15 ml Falcon tubes filled with 2 ml sonication mixture**

- **Important: Wet weight of biological material (Cell or nuclei pellets, embryos etc.) should not be more than 1 g. in 10 ml buffer. Higher concentrations will lead to partially fragmented DNA.**

For sonication, any buffer is suitable, depending on special requirements. A good practice is to use the buffer and extraction procedure already in use with a previous probe sonicator. If you do not have established extraction protocol, refer to section 4.3.6.

**Sonication condition:**

**Bioruptor on “High” (H).**

Fill the tank with cold water (4°C), supplement with 0,5 cm crushed ice.

Sonicate for 15 min total time. (30 sec.”on”, 30 sec. “Off”)

Remove the water in the tank with a cup

Fill again with cold water and crushed ice as above

Sonicate again for 15 min total time. (30 sec.”on”, 30 sec. “Off”).

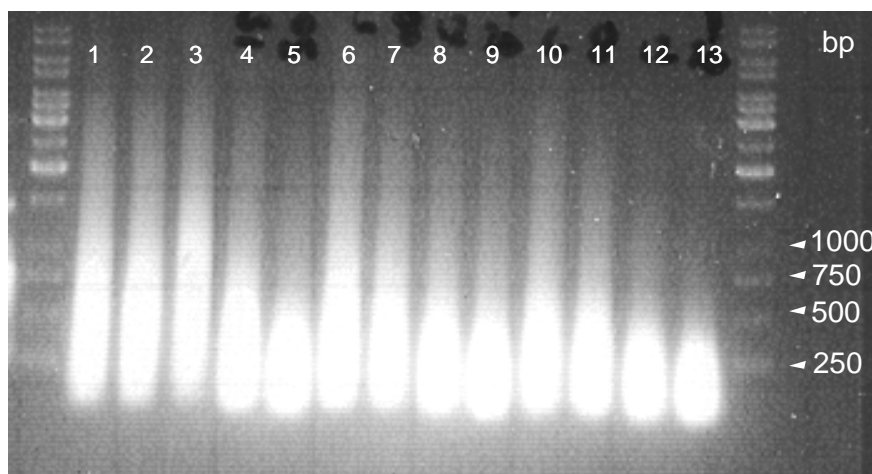
Reverse cross link the sample and analyze on gel electrophoresis.

Small DNA fragments should be obtained as in Fig. 1

Note:

If a high molecular weight DNA smear is observed instead of small 350-200 bp DNA fragments as in fig.1, the amount of starting material (Cell pellet or embryos) is too high and it has to be lowered in the sonication buffer.

Fig. 1: Gel pattern after 30 min. sonication with the Bioruptor (Total time : 30 sec “ON”, 30 sec. “OFF”)



## 4.2 ChIP assay- Sonication of yeast with the Bioruptor

### 4.2.1. Experience from S. Berger's Lab (Wistar Institute, Philadelphia)

A 50ml yeast culture (0.6 to 0.8 OD) is pelleted after the fixation step, which was carried out in 1% formaldehyde for 10 min. at room temperature.

The pellet is beaded (mechanical shearing) following standard procedures to remove cell walls.

The fraction containing chromatin (2 ml in sonication buffer containing SDS or not, depending of the application) is transferred in a 15 ml tube (Corning or Falcon) and closed with the stopper with the metallic bar. The bar tip should not touch the tube walls (In this case Corning 15 ml tubes were used - Cat n°430789).

#### Sonication parameters:

- Bioruptor tank: pre-cooled and filled with cold water supplemented with 0,5 cm floating crushed ice (not more than the blue line, See "getting started" sheet).
- Bioruptor on position "**high**": 30 sec "ON" (red needle), 60 sec "OFF" (green needle);
- Total time: 10 min 30 sec (= 7cycles, = 3min 30 sec cumulative sonication time).  
Temperature of the water bath at the end of sonication procedure is around 10°C

For more precision, the general timer is switched off manually after 10,5 min (General timer on the permanent position, on the left).

The DNA is then electrophoresed on agarose gel after reversing the cross-link.  
DNA fragments size is 250 bp on average, reproducibility between tubes is excellent.

A lot of attention has to be put on cell density, which is crucial to achieve a good reproducibility.

If DNA is not at the right size (or smeared), repeat the experiment either in increasing the total time or in reducing the cell density.

4.2.2. Experience from J. Mellor's Lab (University of Oxford, UK)  
(Dr. A. Morillon, [antonin.morillon@bioch.ox.ac.uk](mailto:antonin.morillon@bioch.ox.ac.uk))

In this protocol, a lot of attention has been put on removing of the cell wall fragments as remaining cell walls in the sonication buffer appears to hamper the fragmentation of DNA by sonication. Furthermore, the sonication step is done at lower power (Medium switch on the Bioruptor).

Yeast cell wall is first digested by Yeast lytic enzyme (YLE from ICN).

The remaining spheroplasts are fixed (1% formaldehyde for 30 min. to one hour, end fixation with glycin), spun down and then beaded (glass beads) following standard procedures (buffer: 1% SDS, 10mM EDTA, 50mM Tris HCl (8.0), 0.5mM PMSF-EtOH, 0.8 g/ml pepstatin A, 0.6 g/ml leupeptin).

After beading, the spheroplast extract is spun down and the supernatant (avoid to disturb the cell debris pellet) is transferred in an Eppendorf tube for sonication with the Bioruptor (300µl per microtube, average cell concentration of  $2 \cdot 10^7$  per ml).

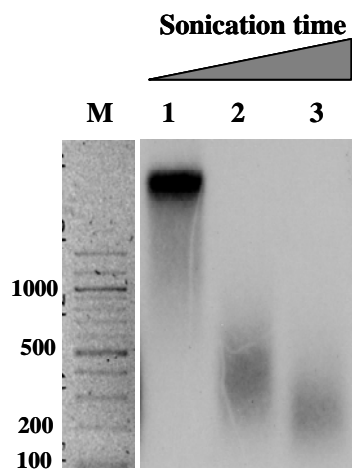
The protocol of sonication has here been optimized to minimize the damage to DNA-associated proteins for the subsequent immunoprecipitation.

Sonication parameters:

- Bioruptor tank: pre-cooled and filled with cold water supplemented with 0,5 cm floating crushed ice (not more than the blue line, See "getting started" sheet).
- Bioruptor on position "**Medium**": 10 sec "ON" (red needle), 20 sec "OFF" (green needle);
- Total time: 16 min 30 sec (= 25cycles, = 4min 10 sec cumulative sonication time).

For more precision, the general timer is switched off manually after 16, 5 min (General timer on the permanent position, on the left).

The DNA is then electrophoresed on agarose gel after reversing the cross-link. DNA fragments size is 350 bp on average ( fig. 1), reproducibility between tubes is excellent.



**Figure 1:** Southern blot experiment to control of the size of sonicated DNA with the Bioruptor.

MET16 locus is revealed by hybridization of a random priming probe (entire gene)  
Sonicated samples were electrophoresed on 1.5% agarose gel.  
Sonication times are 0 (Lane 1), 7 (Lane 2) and 14 minutes (lane 3), respectively.  
M is a 100bp marker (Biolabs) stained with Ethidium bromide.

### 4.3 Sonication of DNA: New data with the Bioruptor

*In Diagenode labs, several experiments have been conducted to evaluate the sonication efficiency of DNA in several conditions with the Bioruptor. This enables to better understanding of the Bioruptor's performances under various circumstances.*

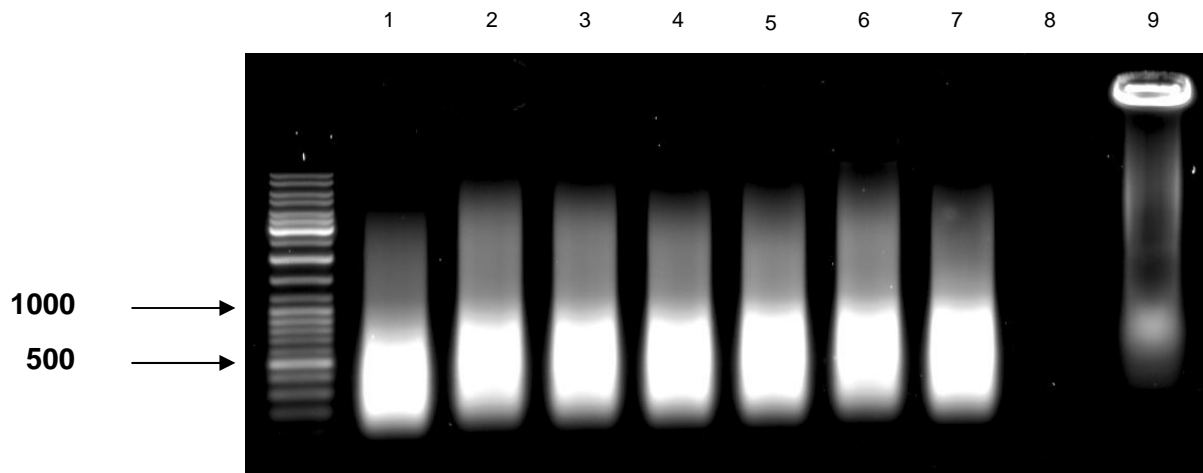
#### 4.3.1 Reproducibility and difference due to the number of tubes sonicated together

This section describes experiments designed to test the reproducibility of the sonication when several tubes are sonicated together in the Bioruptor. Due to the constant rotation during sonication, this reproducibility appears excellent. Additionally, the size of sonicated DNA fragment has been compared when several tubes or a single tube was sonicated at the time. All the experiments were conducted with a standard DNA sample. The complete protocol of cell fixation and extraction is described in section 4.3.6.

##### 4. 3.1.1. Eppendorf format

For the same sonication time, there is a slight difference in fragments size if a tube is sonicated alone in the Bioruptor or if six tubes are sonicated together.

In lane 2 to 7, 6 tubes were inserted together: a regular size distribution of fragment around 500bp is observed. If a single tube is sonicated for the same time (Lane 1), the size of the obtained fragments is slightly lower (around 400bp).



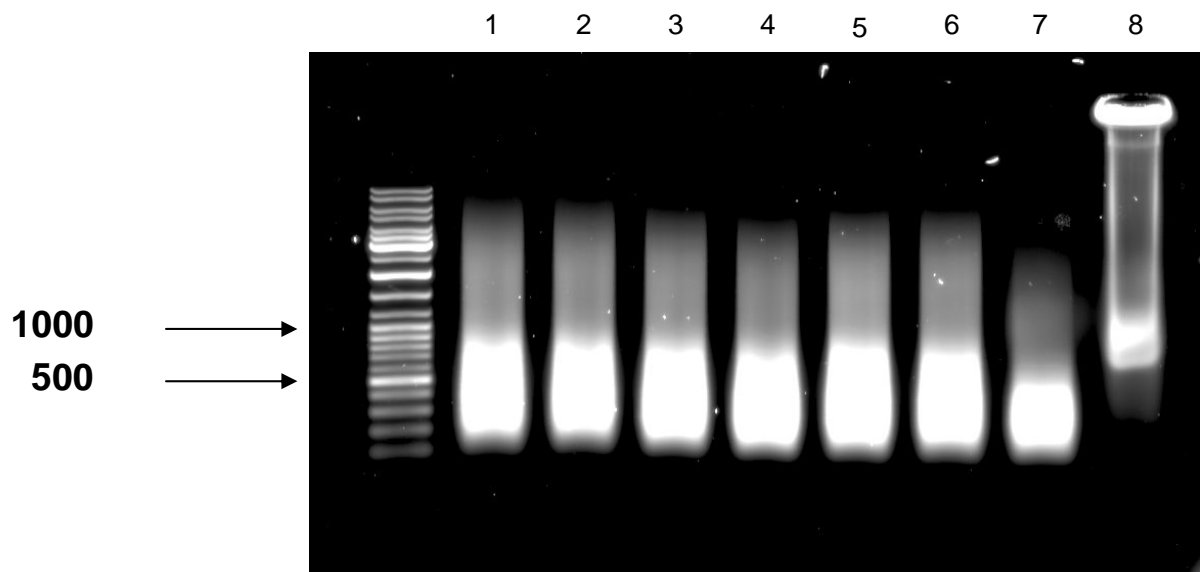
293T cells were fixed with 1, 5 % formaldehyde during five minutes at 37° C.  
Each lane shows purified chromatin fragments from 5.10e6 cells lysed in 300 µl of buffer and sonicated in Eppendorf 1, 5 ml tube.  
Sonication during 5 cycles (30 sec. ON & 30 sec. OFF) at « High » settings.  
First lane shows chromatin from an Eppendorf tube sonicated individually.  
Lanes 2 to 7 show chromatin from 6 tubes sonicated at a time.  
Unsonicated chromatin is shown in lane 9 .



#### 4. 3.1.2. 15 ml tubes format

For the same sonication time, there is a slight difference in fragments size if a tube is sonicated alone in the Bioruptor or if six tubes are sonicated together.

In lane 1 to 6, 6 tubes were inserted together: a regular size distribution of fragment around 500bp is observed. If a single tube is sonicated for the same time (Lane 7), the size of the obtained fragments is slightly lower (around 300bp).



293T cells were fixed with 1,5 % formaldehyde during five minutes at 37° C.  
In each case sonication during 15 **cycles** (30 sec ON & 30 sec. OFF) at « High » settings.  
5 µg of purified chromatin was loaded on a 1% agarose gel.

Each lane show chromatin fragments from 1,5.10e6 cells lysed in 1ml of buffer and sonicated in a Greiner 15ml tube.

Lane 1 to 6 show chromatin fragments from six Greiner tubes sonicated at a time.

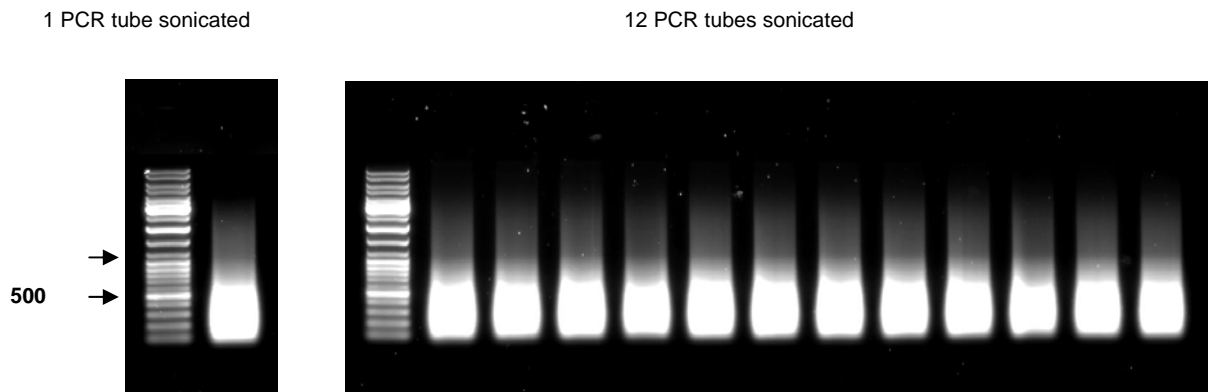
Lane 7 shows fragments from a Greiner tube sonicated individually.

The extreme right lane (8) shows unsonicated chromatin.

We see that the efficiency of sonication is diminished by the presence of six tubes in the water bath.

#### 4. 3.1.3. PCR tubes format

For the same sonication time, a Bioruptor processed 12 tubes together (Right panel) or a single tube individually (Left panel). The results do not show any clear difference in fragment size between the 2 conditions.



293T cells were fixed with 1,5% formaldehyde during five minutes at 37°C.

Each lane shows purified chromatin fragments from 3,3.10e6 cells lysed in 100 µl of buffer and sonicated in 0,5 ml PCR tube.

The left image shows result of a single PCR tube sonicated alone during 5 cycles (30 sec. ON & 30 sec. OFF).

The image on the right shows fragments of chromatin from 12 tubes sonicated together during five cycles.

#### 4. 3.1.4. Conclusion

For the 3 containers tested, an excellent reproducibility has been obtained when sonicating several tubes together.

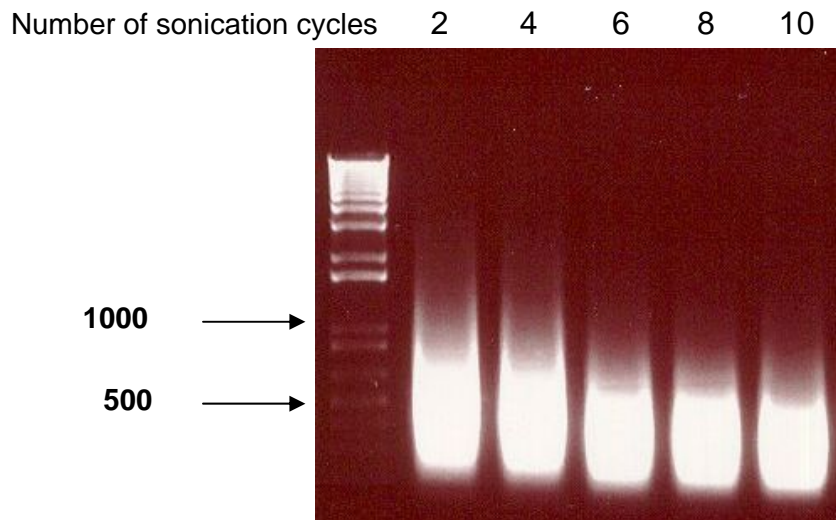
When the sonication was done with a single tube, there is apparently a slight increase of the DNA disruption efficiency, except in the PCR tube format where no difference in the DNA fragment size could be found.

The discrepancy observed with the two other formats can be avoided in using spared empty tubes when sonicating a single sample.

#### 4.3.2 Effect of cumulative sonication cycles on fragment size

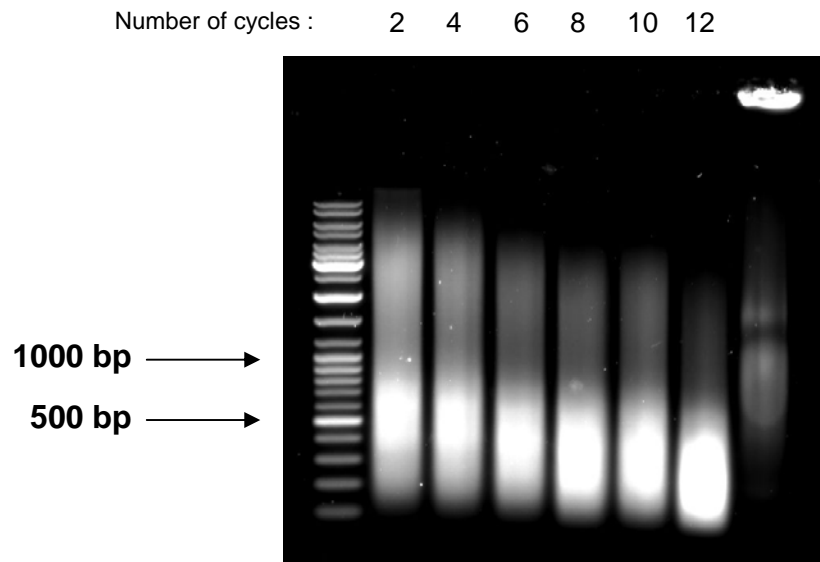
Depending on the required size of DNA fragments, the Bioruptor could be tested during graded sonication times.

#### 4. 3.2.1. Eppendorf format



U2OS cells were fixed with 1.5% formaldehyde for 5 minutes at 37°C.  
Each lane shows purified chromatin fragments from 5.10e6 cells lysed in 300 µl of buffer and sonicated in Eppendorf 1,5 ml tube.  
Sonication during 2, 4, 6, 8 or 10 cycles (30 sec. ON & 30 sec. OFF) at « High » settings.  
5 µg of purified chromatin loaded in each well of a 1% agarose gel.

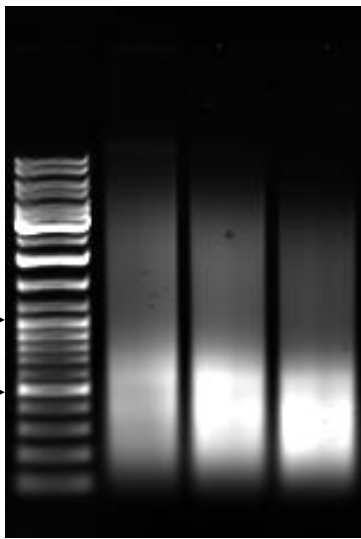
#### 4. 3.2.2. 15 ml tubes format



293T cells were fixed with 1,5 % formaldehyde during five minutes at 37° C.  
Each lane shows purified chromatin fragments from 1,5.10e7 cells lysed in 1 ml of buffer and sonicated in Greiner 15 ml tube.  
Sonication during 2, 4, 6, 8, 10 or 12 cycles (30 sec. ON & 30 sec. OFF).  
5 µg of purified chromatin loaded on a 1% agarose gel.  
The last lane shows unsonicated chromatin.

#### 4. 3.2.3. PCR tubes format

Number of cycles :      1      3      5



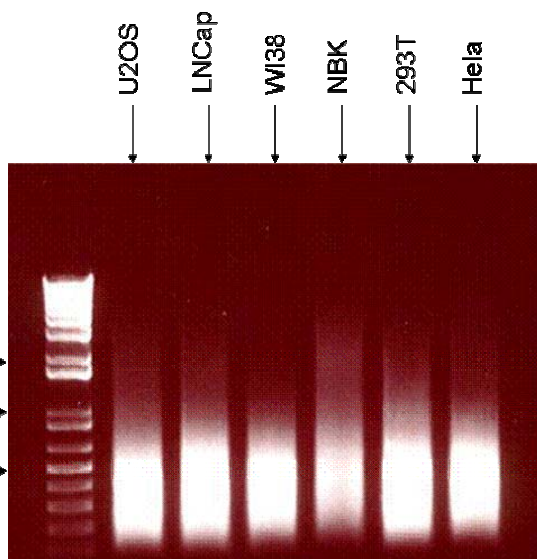
293T cells were fixed with 1,5 % formaldehyde during five minutes at 37° C.  
Each lane shows chromatin fragments from 3,3.10e6 cells lysed in 100 µl of buffer and sonicated in a 0,5 ml PCR tube.  
Sonication during 1, 3 or 5 cycles (30 sec. ON & 30 sec. OFF) individually at « High » settings.  
We achieve good sonication with fragments under 500 bp after 5 cycles.

#### 4. 3.2.4. Conclusion

Typical sonication with the Bioruptor produces DNA fragments of 500 bp and below. If sonicated for shorter times, a smear of larger fragment is observed with progressive enrichment of the 500bp fraction.

#### 4.3.3 Reproducibility with different cell types

The experiments below show similar results when different cell lines were used as starting material for the sonication.



Cells were fixed with 1.5% formaldehyde for 5 minutes at 37°C.

1.10e7 cells of each cell type were lysed in 300 µl of buffer and sonicated in Eppendorf 1,5 ml tube.

Sonication during 5 cycles (30 sec. ON & 30 sec. OFF) at « high » settings.

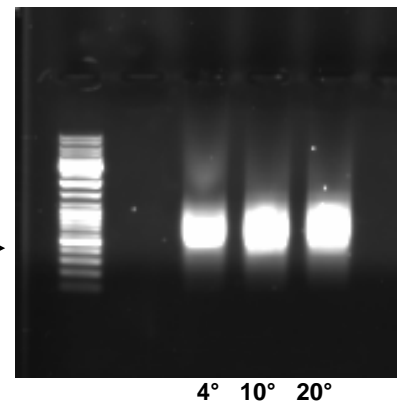
5 µg of purified chromatin loaded in each well of a 1% agarose gel.

#### 4.3.4 Reproducibility at different sonication temperature (Optional cold water circulation system)

When water bath temperature of the Bioruptor was fixed with the cold water circulation system, three tests were conducted at 4°C, 10°C and 20°C to compare the size of the obtained DNA fragments. There is apparently no difference in DNA size, although the conservation of the associated protein might be variable. Additional experiments to test the ChIP signals in these three conditions have to be designed.

293T cells were fixed with 1,5% formaldehyde during 5 minutes at 37°C. Each lane shows purified chromatin fragments from 3,3.10e6 cells lysed in 300 µl of buffer and sonicated in 1,5 ml eppendorf tube. The figure shows a the result of a single Eppendorf tubes sonicated during 5 cycles (30s On / 30s Off ) at different water bath temperatures: 4°C 10°C 20°C.

500 bp →



#### 4.3.5 Reproducibility with different sonication volumes

Experiments were done to compare the result of sonication if Eppendorf tubes or 15 ml tubes were filled with different volumes.

The tested volumes were the maximal sample volume, and a volume representing a quarter from this maximal samples volume.

The results (not illustrated) do not show any difference between the different conditions and the sonication volume has not been shown to have any influence on the size of the DNA fragments.

#### 4.3.6. Standard fixation and extraction procedure for cell lines

Formaldehyde 37% is directly added to cell culture medium to a final concentration of 1,5%. Roll the cell culture dish to mix the Formaldehyde.

The fixation step lasts 5 min. at 37°C.

Cells are then washed two times with cold PBS and then collected with a cell scraper.

The cell pellet is collected by centrifugation (3000g, 5min. 4°C)), resuspended in 1 ml Buffer A ( all volumes are given for confluent dish of 15 cm diameter, around 10 millions cells) and incubated 15min. on ice, then 15 min. at 30°C.

Vortex briefly and centrifuge as above.

For the lysis step,

- The cell pellet is resuspended in 1 ml buffer B.

- Incubate 5 min. at 4°C and spin as above.
- The cell pellet is resuspended in 1 ml buffer C.
- Incubate 5 min. at 4°C and spin as above.
- The cell pellet is resuspended in 300µl buffer D.

**Sonication** in Eppendorf tubes. 30 sec “On”/ 30 sec. “Off”, 5 min total time (5 cycles). Spin as above.

The supernatant can be tested directly on agarose gel to test the DNA disruption (10 µl/lane on 1% agarose gel).

However, the gel electrophoresis of these crosslinked samples in SDS buffer often gives smeared pictures.

For accurate size determination of the DNA fragments, the reversion of the cross-linking and DNA precipitation after Phenol/chloroform extraction is advised.

#### **Buffer A :**

100 Mm Tris-HCl à pH 9,4  
10 Mm DTT

#### **Buffer B :**

10 mM EDTA  
0,5 mM EGTA  
10 mM HEPES PH 6,5  
0,25% Triton X-100

#### **Buffer C :**

10 Mm EDTA  
0,5 mM EGTA  
10 mM HEPES PH 6,5  
200 mM NaCl

#### **Buffer D :**

10 mM EDTA  
50 mM Tris-HCl pH 8  
1% SDS (BIORAD, #161-0302 (1kg), the quality of the SDS seems to be critical)  
0,5 % Empigen BB (Protease inhibitor)

### **Reversion Protocol**

50 µl of the sonicated supernatant is mixed with 400 µl of buffer D and 5µL of proteinase K (10mg /ml).

Incubate at least 4 hours at 65°C. (overnight is OK).

Add 455 µl of phenol / chloroform / isoamyl alcohol mixture

Vortex  
Centrifuge 5 min at max speed.  
Collect the supernatant.  
Add 400µl chloroform  
Vortex  
Centrifuge 5 min at max speed  
Collect the supernatant.

Precipitate with NaAC with glycogene.  
Resuspend final pellet in 50 µl water or TE.  
Analyze on 1% agarose gel (10µl sample/lane).

#### 4.3.7. Tips to obtain quality electrophoresis pictures

The migration of large quantities of DNA on agarose gel can lead to poor quality pictures which do not reflect the real DNA fragmentation.

The important factors are:

- Gel overloading: do not load more than 5 µg/lane
- Agarose concentration: do not use more than 1% agarose gel and run slowly (not more than 100volt)
- Running buffer concentration: 1X TAE or TBE is preferred to 0,5X TAE which can lead to smeared gels (Minigel migration).
- SDS quality in the sonication buffer is very important.  
A SDS brand which is working OK is Biorad (Cat #161-0302 - 1kg).
- Reversion of the cross-linking: To obtain clear image with accurate fragment size.