PROTOCOL

# RNA Shearing for Bioruptor® NGS

## Operating conditions

**Sample volume:** 100 µl

**Tubes:** Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat. No. WA-005-0500)

**Tube holder:** 0.5/0.65 ml tube holder (Cat. No. UCD-pack 0.5) for 12 x 0.65 ml tubes

**Sonication buffer:** TE (10 mM Tris, 1mM EDTA), pH 7.5 - 8.0), RNase free

**RNA concentration:** 0.05 µg/µl

Samples are vortexed (briefly) and centrifuged (10 sec) before shearing.

**For optimal results samples should be stored on ice during 10-15 minutes prior to sonication. In addition, short centrifugation steps after every 5 cycles are highly recommended.**

**Temperature:** 4°C – Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) & Connector kit for Water Cooler (Cat. No. VB-100-0001)

**Power setting:** H position (High)

**Sonication cycle & total sonication time:** varies depending on desired RNA size

**Note:** Recommended protocols are subject to change without notice. Additional protocols are available on demand.

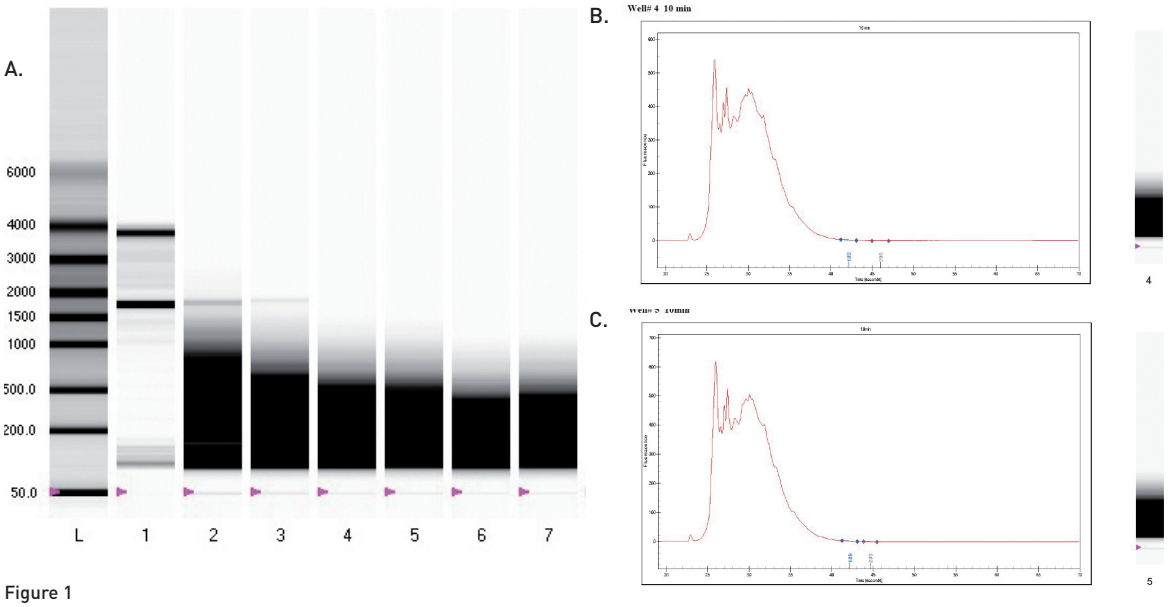


Figure 1

***Programmable RNA size distribution and excellent reproducibility with Bioruptor® NGS***

*The various panels show different RNA size distributions of sheared total RNA produced by varying the duration of sonication on the Bioruptor® NGS. Panel A shows duplicate profiles produced after 5 (lanes 2-3), 10 (lanes 4-5) and 15 minutes (lanes 6-7) (30 sec on/off) of sonication. Lane 1 shows the unfragmented total RNA (starting material). Panel B and C compare the RNA size distributions of sheared total RNA from 2 different experiments.*

*All samples were analysed on Biorad Experion using Eukaryote Total RNA HighSens chip.*

*Panel A: gel virtual view (all lanes). Panel B and C: peak electropherogram view + gel virtual view (one lane each).*

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## General remarks on handling RNA

When working with RNA, care must be taken to maintain an RNase-free environment starting with RNA purification and continuing through analysis. Wear gloves at all times to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep isolated RNA on ice when aliquots are pipetted for downstream applications. Use sterile technique when handling the reagents used for RNA isolation, shearing or analysis. Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases that works by covalently modifying RNases.

## Important comments about RNA shearing

The Diagenode ACT (Adaptative Cavitation Transfer technology) process is highly reproducible, however attention must be paid to the following treatment attributes to ensure best results:

- **Tubes:** At present, the recommended tube vessels are the Diagenode's Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat. No. WA-005-0500). These tubes are also valid for RNA shearing and are RNAse free. Pay attention not to damage the cap when closing the tubes since this could alter sonication results.

- **Sample volume:** The recommended volume of the Diagenode's Bioruptor® NGS 0.65 ml Microtubes for DNA Shearing (Cat. No. WA-005-0500) is 100 µl. When using lower volumes (eg. ≤ 50 µl), less reproducible results may be observed due to an alteration of the ultrasonic waves distribution in the sample fluid; thus, reducing the efficiency of sonication which may result in broader size distribution or larger peaks.

- **Sample concentration:** Diagenode recommends using RNA concentration ranging between 1 and 20 ng/µl (10 ng/µl recommended). Using larger concentration (eg. 50-100 ng/µl) may result in broader peaks or variable peak distribution.

- **Sample preparation:** Sample viscosity may have a major impact on sonication results. Careful resuspension of RNA sample is strongly recommended before sonication processing. Multiple pipetting and gentle vortexing followed by a short centrifugation to recover sample volume at the bottom of the tube is therefore strongly recommended. Storing RNA samples on ice during 10-15 minutes before sonication has also been shown to improve reproducibility.

- **RNA quality:** RNA quality and quantity must be considered carefully since bad quality and quantity RNA may have several impacts on sonication and next-gen sequencing downstream applications. Therefore it is highly recommended to use only high quality RNA when sonicating RNA.

- **Water temperature:** Propagation of ultrasound in a liquid unavoidably produces heat that can ultimately alter RNA sample (eg. by thermal denaturation). To ensure the best preservation of the sample, it is recommended to start the sonication process with cold water in the water bath. During sonication, especially when doing long sonication runs, the temperature must also be controlled. This is obtained by the automatic temperature control.

**Note:** The permanent installation of the Bioruptor® in a cold room is possible, although not sufficient to avoid the temperature increase due to sonication.

- **Automatic temperature control:** A recirculating Bioruptor® Water Cooler is used to guarantee the automatic temperature control of the water bath during the whole sonication process. This Bioruptor® Water Cooler (Cat. No. BioAcc-cool) produces a regular water flow with a constant water level in the tank. An additional

regulating valve (Single Cyvle Valve for Bioruptor® Water Cooler, Cat. No. VB-100-0001) ensures that water will only be replaced during the off cycle to avoid any interference between water flow and the sonication process.

- **Sonication time:** Minor adjustments in cycle number may be made to optimize results for various sample types and concentrations. Cycle number listed above is a recommended guideline. Actual results may vary depending on the amount and type of starting material, concentration, viscosity and/or plastic tubes. Diagenode recommends setting up a time dose response experiment for determining appropriate cycle number. Higher concentration may require a longer dose to ensure a homogeneous shearing result.

- **Water bath:** The sonication water bath is a critical component of the Bioruptor® sonication system.

    1. **Water purity:** Contaminants such as algae and particules may alter the ultrasonic waves propagation, resulting in broader size distribution or larger peaks. Bath water should be pure distilled water, changed regularly.
    2. **Water bath maintenance:** The water bath metal surface is fragile and requires a careful maintenance. Use only soft sponge to remove traces. Never use scratch scrub sponge since this would alter the ultrasonic wave emitter surface.

**Supplementary Data:**

Please note that there are three main sources of variation in both peak base-pair size and distribution:

1) The physical process of RNA fragmentation might not be entirely random.

2) The analytical process to determine fragment size has inherent variances (for example, gel electrophoresis and microfluidics-based platform). Therefore, fragment distributions and peak values, even from technical replicates, may not appear identical.