PROTOCOL

Bacterial Cell Disruption using Bioruptor® Standard, Bioruptor® Plus or Bioruptor® XL

For cell lysis, we highly recommend using 1.5 ml TPX microtubes (Cat. No. M-50050) or 10 ml tubes (Cat. No. AS-100) and the corresponding tube holders (Cat. No. UCD-pack 1.5 and UCD-pack10). To guarantee homogeneity of sonication, the tube holders should always be completely filled with tubes.

Operating conditions:

Tubes: 1.5 ml TPX microtubes or 10 ml tubes

Tube holder: 1.5 ml tube holder (Cat. No. UCD-pack 1.5) or 10 ml tubes holder (Cat. No. UCD-pack 10) with reflecting bar

Sample volume: 300 µl for 1.5 ml TPX microtubes
2 ml for 10 ml tubes

Sonication buffer: PBS with protease inhibitor cocktail

Temperature: Maintain at 4°C by using the Bioruptor® Water Cooler (Cat. No. BioAcc-Cool) or by using crushed ice

Power setting: H position (High)

Sonication cycle: 30 sec ON, 30 sec OFF

Total sonication time: 10 min for UCD200/300
15 min for Bioruptor XL

Note: *Please note that additional optimization might be required depending on the bacterial strain and growth phase. Gram-positive bacteria are more resistant to sonication than Gram-negative bacteria because of the rigid cell wall. Cells in log phase are less resistant than cells in stationary phase. In order to preserve protein structure and activity, avoid a long sonication.*

Protocol:

1. Collect cells by centrifugation at 1000 g for 10 min at 4°C
2. Wash twice with cold PBS.
3. Resuspend cells in cold PBS to OD600 3.0.
4. Transfer cell suspension to sonication tubes. For optimal efficiency, use the recommended sample volume.
5. Sonicate at High Power for 10 min (UCD200/300) or 15 min (Bioruptor® XL)
6. Centrifuge at 15.000 rpm for 15 min at 4°C.
7. Separate the soluble fraction (supernatant) from the insoluble (pellet).
8. The pellet can be used for extraction of insoluble proteins with a denaturating buffer of choice.

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Efficient cell disruption with Bioruptor®

Cell suspensions were sonicated for different periods of time ranging from 5 to 20 minutes. Two types of tubes were tested: Diagenode's 1.5 ml TPX tubes (M-50001) and Diagenode's 10 ml tubes (AS-100). The efficiency of cell disruption was initially determined by measuring optical density at 600 nm. The results indicated that the number of intact cells decreases rapidly with increasing sonication time. After only 5 minutes of sonication, a significant number of cells were disrupted (Fig.1). Similar results were observed using the Live/Dead BacLight kit (data not shown) which allows the quantification of live cells with intact membranes and discrimination from cells with damaged membranes. Thus, efficient cell disruption is observed after 5-10 minutes of sonication.

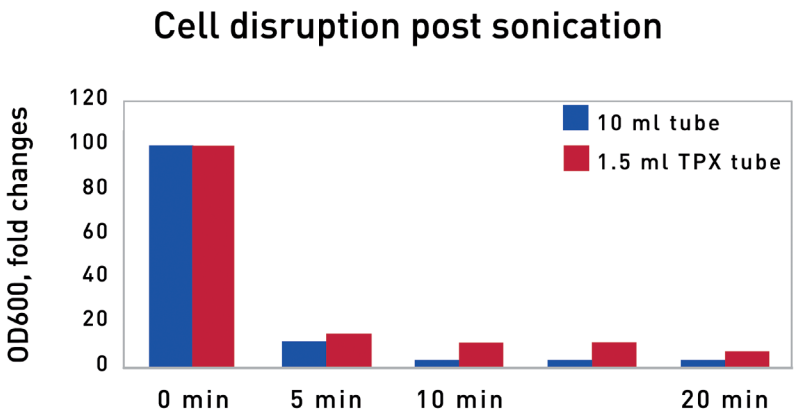


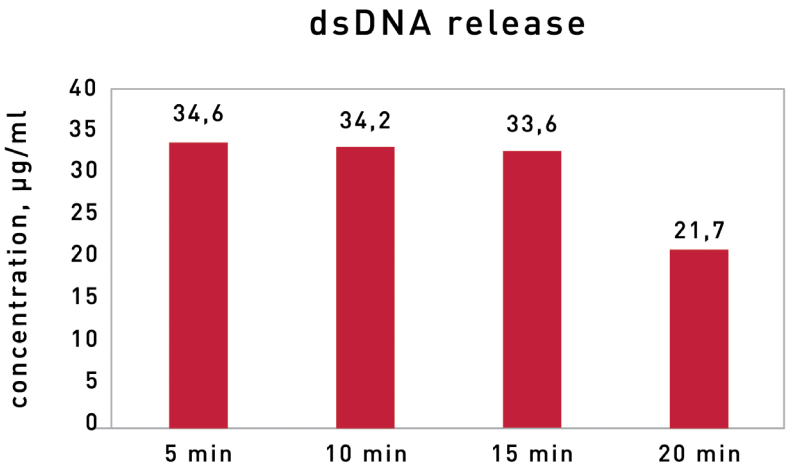
Figure 1: Effect of sonication on cell disruption
The number of intact cells after sonication was determined by measuring optical density at 600 nm. Optical density of the cell culture before sonication (0 min) is arbitrarily set to 100%.

PROTOCOL

Sheared DNA is released during bacterial sonication

The disruption of bacterial cells by sonication releases DNA with maximum recovery after only 5 minutes of treatment (Fig.2, A). The released DNA is fragmented with fragment size dependent on sonication time (Fig.2, B).

A.



B.

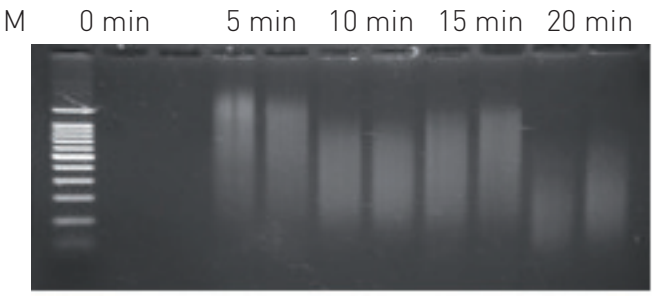


Figure 2 : Effect of sonication on DNA release

Figure A: The DNA concentration in each sample after sonication was quantified with the DNA BR assay kit (Invitrogen)

Figure B: An aliquot of each sample before (0 min) and after sonication was run in a 1.5% agarose gel stained with SybrSafe and visualized in UV light. Lane M represents a 100 bp ladder.