Evaluation and benchmarking of the BeatBox for high-throughput cell lysis

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Introduction

The BeatBox technology is a novel approach for sample homogenization, developed to enhance the lysis and protein extraction steps in proteomic sample preparation protocols. Effective homogenization of biological samples, such as tissue and cells is crucial to extracting and therefore subsequently identifying and quantifying proteins. Extracted proteins can be seamlessly processed using the already established iST technology followed by LC-MS/MS, to perform proteomic analyses and assess the biology of the system being investigated. Traditionally, homogenization/lysis is performed using harsh physical methods, such as sonication, repetitive pipetting, manual grinding, boiling, and bead milling, sometimes combined with freeze/thaw cycles[1]. However, these techniques suffer from various drawbacks. Protein extraction can be inefficient, time-consuming, and prone to cross-contamination. In addition, heat generation induced in the sample can lead to protein degradation. The BeatBox technology overcomes these challenges by enabling simultaneous lysis of up to 96 samples in only 10 minutes, with reliable protein extraction, resulting in the reproducible preparation of large cohorts of samples, such as would be desirable for high-throughput studies[2,3]. In this study, the BeatBox technology was used to process mammalian, yeast, and gram-negative bacterial cells, to evaluate various parameters, such as access to cell membranes and cell wall proteins, inherently and historically difficult to extract. We compared the efficiency of protein extraction of the BeatBox technology to traditional sonication, as well as evaluated different numbers of cells as input material. Downstream proteomic sample preparation was performed using the iST workflow followed by LC-MS/MS analyses (Figure 1).

Keywords

Protein analysis, Cell lysis, Sample preparation, high-throughput homogenization, BeatBox, HEK293 cells, *E. coli*, and *S. cerevisiae*

Key takeaways

The BeatBox is a high-throughput and easy-to-use instrument for cell disruption and tissue homogenization

Efficacious protein extraction and high protein identification rate from various cell types were achieved by BeatBox technology

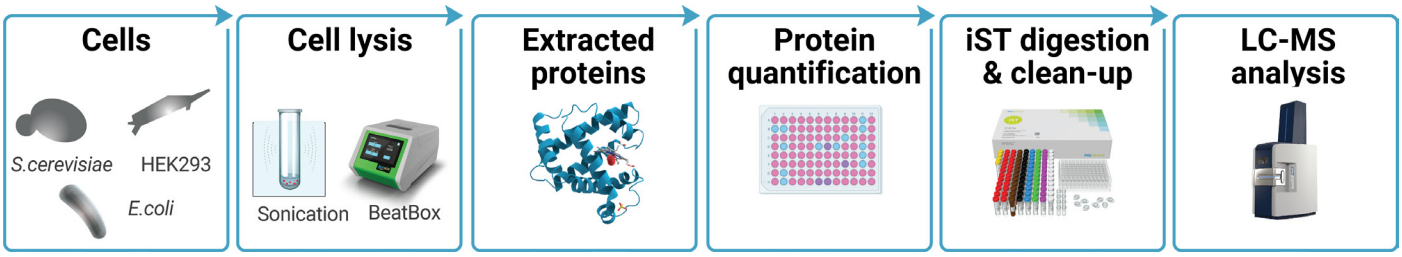

Figure 1 | Full workflow from cell lysis to protein identification.

Material and Methods

The lysis and extraction of proteins from human embryonic kidney cells (HEK293), *Escherichia coli* (*E. coli*), and *Saccharomyces cerevisiae* (*S. cerevisiae*) were compared using the BeatBox-iST sample preparation workflow alongside a sonication-iST workflow. Harvested cells were washed one time in 1X Phosphate Buffered Saline (PBS), aliquoted, and pelleted before the experiment. Two cell counts were tested in combination with BeatBox lysis for every organism: HEK293 (1E5 and 4E5cells), *E. coli* (2.1E7 and 8.4E7 cells), and *S. cerevisiae* (1.1E6 and 4.4E6 cells). Only the higher cell count was processed using sonication-iST workflow.

In the BeatBox workflow, for S. cerevisiae only, the samples were transferred to the BeatBox tissue kit plate. The cells were resuspended in 100 µL of LYSE buffer and boiled for 10 min at 95°C, 1 000 rpm, and then cooled. Then *E. coli* and HEK293 cells were transferred to the BeatBox tissue kit plate. They were resuspended in 100 µL of LYSE buffer. Then all three cell types were lysed for 10 min at room temperature using a standard BeatBox setting. For the sonication workflow, cells were resuspended in 50 µL of LYSE buffer and boiled at 10 min, 95°C, and 1 000 rpm. After cooling down, the samples were sonicated for 10 min at 4°C (30 sec ON, 30 sec OFF).

For protein quantification, a few microliters of the resultant lysates were diluted in 1X PBS (1:100) and measured using a Micro BCA™ Protein Assay (ThermoFisher Scientific). All samples were prepared for LC-MS/MS using the iST kit for denaturation, reduction, alkylation, digestion, and peptide cleanup. Prior to LC-MS/MS analysis, peptide concentrations were determined using NanoDrop™ (ThermoFisher Scientific).

Peptides (300 ng) were analyzed in a short 45-minute run on an EASY-nLC™ 1200 system (ThermoFisher Scientific) coupled with a timsTOF Pro (Bruker Daltonics) in DDA PASEF mode[3]. Data analysis was performed using MaxQuant (v. 2.0.1.0), with the searches carried against the corresponding species specific database ((Human up000005640, *E. coli* up000000625, *S. cerevisiae* up000002311 (download date 17/03/2022)). The search settings controlled for a 1% FDR at both the peptide and protein level, using a decoy database of reverse sequences, and proteins were retained for analysis with a minimum of one unique peptide. The search for the Gene Ontology term enrichment was performed using STRING (v11.5) and data were filtered with a score of ≥ 0.9 and a protein 1%FDR.

Results and Discussion

The aim of this study was to demonstrate that the BeatBox can lyse different cell types efficiently and show that it achieves equivalent or better performance in protein yield and protein groups identified when compared to the established cell disruption technique of sonication. The protein yields assessed using the protein assay give information about the cell lysis efficiency and protein extraction using BeatBox and sonication (Figure 2).

For *E. coli* and *S. cerevisiae* samples, total protein levels measured by BCA assay showed higher protein yields using BeatBox than sonication. In contrast, for HEK293 samples, lower protein yields were calculated using BeatBox compared to sonication. However, a comparison of the number of proteins showed the identification of more protein groups using BeatBox than sonication for this cell type (Figure 3).

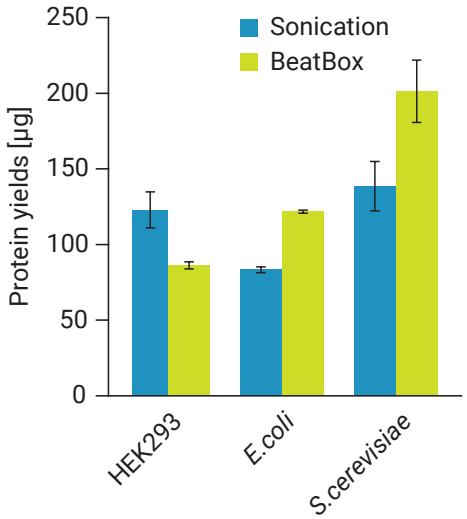

Figure 2 | Comparison of the amount of protein extracted using sonication and BeatBox on HEK293 cells (4E5 cells), *E. coli* (8.4E7 cells), and *S. cerevisiae* (4.4E6 cells).

Harvested cells were washed in 1X PBS, aliquoted, and pelleted before the experiment. The total volume of LYSE buffer was 50 uL and 100 µL for sonication and BeatBox, respectively. Samples were prepared in triplicates, the error bars represent the standard deviation.

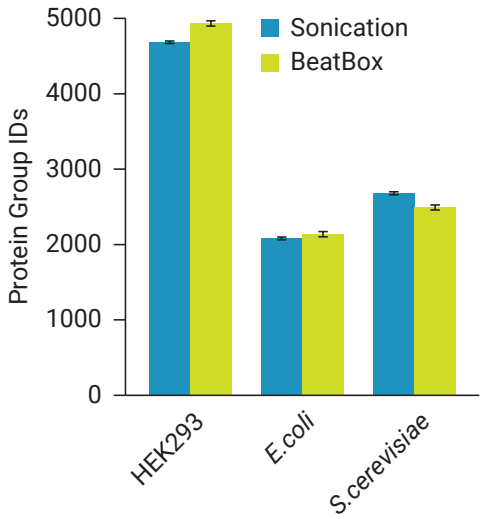

Figure 3 | Comparison of the number of protein groups identified using BeatBox and sonication on HEK293 cells (4E5 cells), *E. coli* (8.4E7 cells), and *S. cerevisiae* (4.4E6 cells).

300 ng of peptides were used for all experiments to perform the LC-MS/MS analysis. Samples were prepared in triplicates, the error bars represent the standard deviation.

BeatBox homogenization with four times lower cell inputs resulted in ~4 300 protein groups for 1.0E5 HEK293 cells, ~2 000 protein groups from 2.1E7 E. coli cells, and ~2 500 protein groups from 1.1E6 *S. cerevisiae* cells. In comparison with a higher number of cells (Figure 3), the number of proteins is comparable due to the equal amount of peptides loaded on the column (300 ng). The repeatability of protein group numbers for low and high input of cells using both workflows was less than 3%. The results illustrate the proficiency of the BeatBox to lyse various cell types with varying counts of cells in a high-throughput manner.

Figure 4 shows the overlap of identified proteins between the two cell disruption techniques. Both approaches recovered similar proteomes for each cell type. Interestingly, more differences were observed for HEK293 cells, where Gene Ontology (GO) analysis revealed enrichment of specific cellular components when BeatBox is used for cell disruption (Table 1).

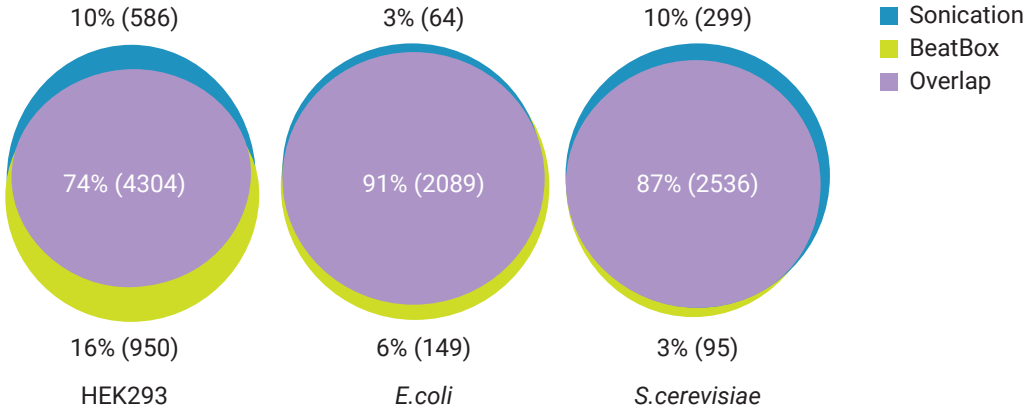

Figure 4 | Venn diagram of proteins group identifications using BeatBox and sonication on HEK293 cells (4E5 cells), *E. coli* (8.4E7 cells), and *S. cerevisiae* (4.4E6 cells).

The numbers for each lysis workflow/cell types conditions correspond to the total number of protein groups among the three sample preparation replicates.

Table 1 | Table of GO-enriched cellular components obtained from BeatBox and sonication protein groups in HEK293 cells.

The search was performed using STRING (v11.5) and data were filtered with a score of ≥0.9 and a protein FDR of 1%. Between parenthesis is the number of observed gene counts per GO among the three sample preparation replicates.

Shared GO Terms	BeatBox exclusive GO Terms	Sonication exclusive GO Terms
Intracellular (BeatBox: 768, Sonication: 459)	Organelle membrane (222)	Catalytic complex (62)
Intracellular organelle (BeatBox: 685, Sonication: 418)	Microtubule cytoskeleton (96)	Autophagosome (11)
Organelle (BeatBox: 721, Sonication: 443)	Microtubule organizing center (64)	
Intracellular membrane-bounded organelle (BeatBox: 611, Sonication: 375)	Centrosome (51)	
Membrane-bounded organelle (BeatBox: 671, Sonication: 417)	Spindle (37)	
Cytoplasm (BeatBox: 626, Sonication: 359)	TRAPP complex (7)	
Nucleus (BeatBox: 417, Sonication: 275)	Endosome (71)	
Nucleoplasm (BeatBox: 251, Sonication: 172)	Ubiquitin ligase complex (29)	
Cytosol (BeatBox: 302, Sonication: 191)	Endomembrane system (252)	
Nuclear lumen (BeatBox: 274, Sonication: 188)	Bounding membrane of organelle (131)	
Protein-containing complex (BeatBox: 290, Sonication: 188)	Organelle envelope (83)	
Cellular anatomical entity (BeatBox: 830, Sonication: 488)	Vesicle tethering complex (11)	
Transferase complex (BeatBox: 59, Sonication: 40)	Lysosomal membrane (34)	
Intracellular organelle lumen (BeatBox: 316, Sonication: 223)		

GO analysis showed that 48% of the enriched GO terms are common to both lysis processes despite independent protein identifications. Thirteen GO terms were highlighted as specifically enriched by the BeatBox protocol compared to just two for the sonication. Moreover, amongst the BeatBox-exclusive GO terms were many cellular components linked with membrane-associated localization of proteins. The physical-chemical properties of peptides were analyzed to confirm indications regarding protein localization in cells (Figure 5).

No significant differences in the properties of the peptides belonging to *E. coli* and *S. cerevisiae* were observed. However, peptides from HEK293 cells have a higher hydrophobicity, as illustrated by a higher GRAVY median, and a very different isoelectric point profile with a median close to neutral pH, which is generally accepted to be associated with membrane localization of proteins[4]. This aligns with the GO analysis, where more proteins were associated with a membrane localization cellular component when BeatBox was used. Membrane localization at the interface of different cellular compartments is of great biological importance. Proteins at such interfaces enable the transport of other key components in cell signalling, making them keystones of many biological pathways[5] . The findings of this study with HEK293 cells are of great interest, because the membrane localization of proteins usually hinders their effective extraction and subsequent detection by MS, leaving them under-represented in the resulting analyses. The potential shown here for BeatBox to gain access to this subset of biologically interesting and challenging proteins, whilst not compromising on the accessibility of other cellular components compared to traditional extraction methodologies warrants further investigation and will be the subject of more in-depth investigation in the future.

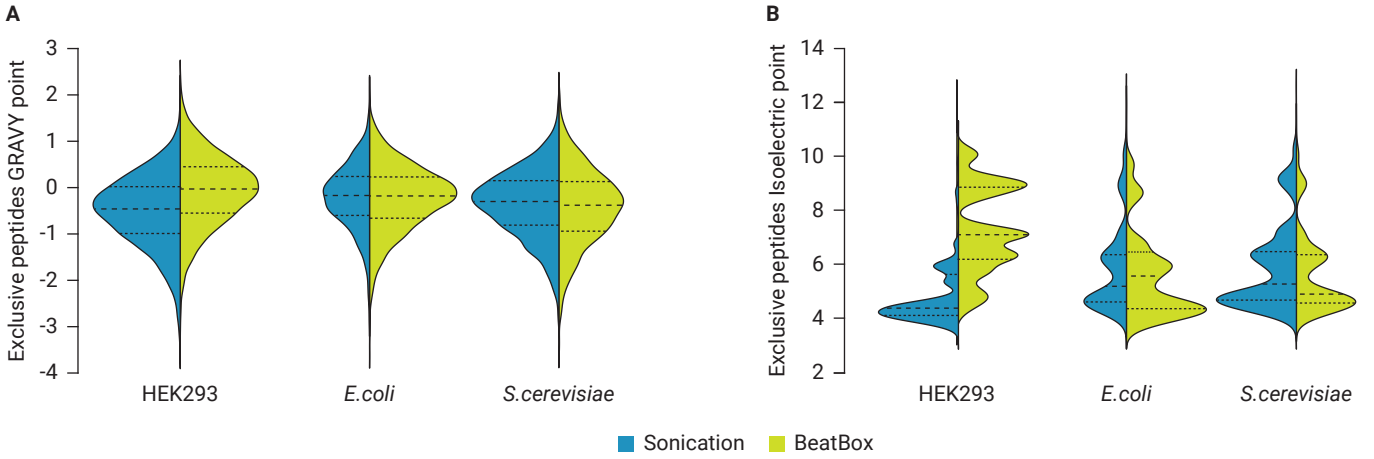

Figure 5 | Violin plot of the GRAVY (A.) and isoelectric point (B.) of peptides exclusively identified using BeatBox or sonication.
For HEK293 cells, 13 239 peptides were BeatBox specific, 14 740 were sonication specific and 25 931 peptides were shared. For *E. coli*, 3 650 peptides were BeatBox specific, 1 939 were sonication specific and 16 929 were shared. For *S. cerevisiae*, 2 836 peptides were BeatBox specific, 3 825 were sonication specific and 17 104 were shared. The width of the plots depends on the peptide counts and is not comparable between species for readability reasons.

Conclusions

The BeatBox is a powerful, versatile, and straightforward solution for homogenizing diverse cell types. Combined with the well-established iST workflow for proteomic sample preparation, it achieves protein yields and identifications equivalent to or better than the sonication pipeline. In mammalian cells, the BeatBox workflow appears to show enrichment of membrane proteins that form a foundation of biological pathways due to their specific localization and are known to be challenging to recover with conventional proteomics sample preparation protocols. Finally, a key advantage of the BeatBox is its ability to homogenize 96 samples in ten minutes, without the risk of cross-contamination and at a constant temperature, making it ideal for high-throughput sample preparation.

Products

Product	Manufacturer	Product Code
BeatBox Instrument	PreOmics GmbH	P.O.00103
BeatBox Tissue Kit 96x	PreOmics GmbH	P.O.00121
iST 96x	PreOmics GmbH	P.O.00027

Ordering information:

http://www.preomics.com/quote

order@preomics.com

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