Determining Efficiency of BeatBox and Sonication Methods for Protein Extraction Using Mass Spectrometry Analysis

Valerie J. Barrera Estrada[1], Carly A.I. Twigg[3], Jesenia M. Perez[2], and Stefani N. Thomas[3]

[1]College of Biological Sciences, [2]Microbiology, Immunology, and Cancer Biology Program, [3]Laboratory Medicine and Pathology

University of Minnesota

Introduction

- Physical disruption and reagent-based methods are used to extract proteins from tissues or cells prepared for mass spectrometry analysis in a process known as cell lysis.[4]
- Sonication is commonly used for protein extraction as it disrupts the cell membrane.[3]
- BeatBox, a device offered by PreOmics™, uses single magnetic particles with proprietary coating that prevents protein binding, called gyuto beads.
- Gyuto beads homogenize and lyse biological samples, and are advertised as being able to efficiently extract proteins for mass spectrometry analysis.
- Efficient protein extraction during sample preparation results in increased protein identifications and more accurate protein-level quantifications following mass spectrometry workflows.
- Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is commonly applied to analyze complex samples and protein mixtures, including biological samples.[1,2]
- **We hypothesize sample preparation with BeatBox will yield a higher number of protein identifications compared to using the conventional sonication method.**

Methods

- Protein digestion was performed using trypsin to obtain tryptic peptides.
- Samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Q Exactive Plus mass spectrometer (n = 2 injection replicates)
- For protein, peptide, and Peptide Spectrum Match (PSM) identification, Proteome Discoverer 2.5 (PD) with SEQUEST was used.
- A T-test was performed using GraphPad Prism v.9 to compare the protein, peptide, and PSM identification between the two methods.
- A One-way ANOVA and Tukey HSD test compared the processing replicates of each method to each other.

References

1. **Aebersold, R., & Mann, M.** (2003b). Mass spectrometry-based proteomics. Nature, 422(6928), 198–207. https://doi.org/10.1038/nature01511
2. **Jiang, X., Jiang, X., Han, G., Ye, M., & Zou, H.** (2007). Optimization of filtering criterion for SEQUEST database searching to improve proteome coverage in shotgun proteomics. BMC Bioinformatics, 8(1). https://doi.org/10.1186/1471-2105-8-323
3. **Kwon, Y. T., & Jewett, M. C.** (2015). High-throughput preparation methods of crude extract for robust cell-free protein synthesis. Scientific Reports, 5(1). https://doi.org/10.1038/srep08663
4. **Rogers, J. A., & Bomgarden, R.** (2016). Sample Preparation for Mass Spectrometry-Based Proteomics; from Proteomes to Peptides. Advances in Experimental Medicine and Biology, 43–62. https://doi.org/10.1007/978-3-319-41448-5_3

Acknowledgements

This research was supported and funded by the NIH, NCATS UL1TR002494 (University of Minnesota Clinical and Translational Science Institute) and the V Foundation for Cancer Research.

Experimental Design & Main Findings

1. Steps conducted for the analysis of the lysis methods using mass spectrometry

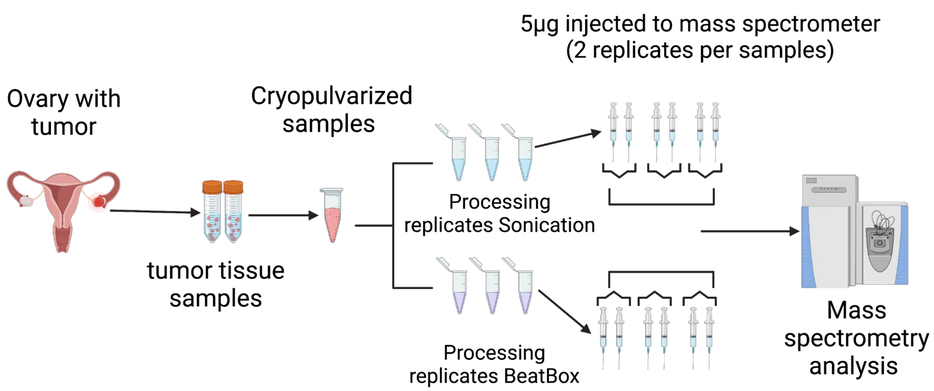

Figure 1: Two ovarian tumor tissue samples were cryopulverized and pooled. Three processing replicates for each lysis method used were prepared; 5µg of the sample was injected into the mass spectrometer for analysis (2 injections made for each sample).

2. 35.4% of the shared proteins were up-regulated using the BeatBox method

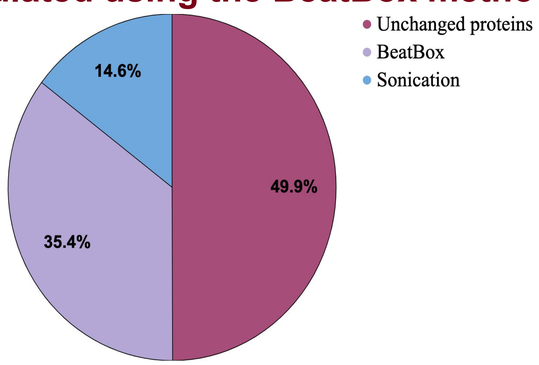

Figure 2: In total, 983 proteins were shared between BeatBox and Sonication lysis methods. A Welch's t-test was performed comparing the two methods and used to identify proteins with significantly higher abundances. For the BeatBox method, 348 proteins were found to be up-regulated ($p < 0.05$); in the Sonication method, 144 proteins were up-regulated ($p < 0.05$). 50% of the proteins (491) had similar protein abundances between the two methods used. Overall higher protein abundances were seen using the BeatBox compared to Sonication.

3. Lack of technical reproducibility using the BeatBox and Sonication lysis methods

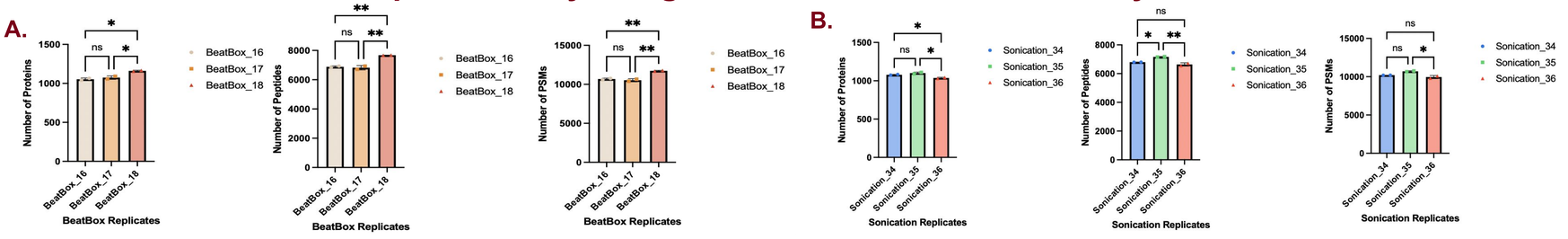

Figure 3: BeatBox samples and Sonication samples were separately compared using a one-way ANOVA test to find differences between protein, peptide, and PSM identification. A) In the BeatBox method, A Tukey HSD test identified sample BeatBox_18 to have significantly higher protein, peptides, and PSM identification compared to the other samples. **B)** In the Sonication method, A Tukey HSD test identified sample Sonication_36 to have significantly lower protein identification, while sample Sonication_35 had higher peptide identification, and higher PSM identification compared to sample Sonication_36.

4. No significant difference in protein, peptide, or PSM identification is seen between the two methods

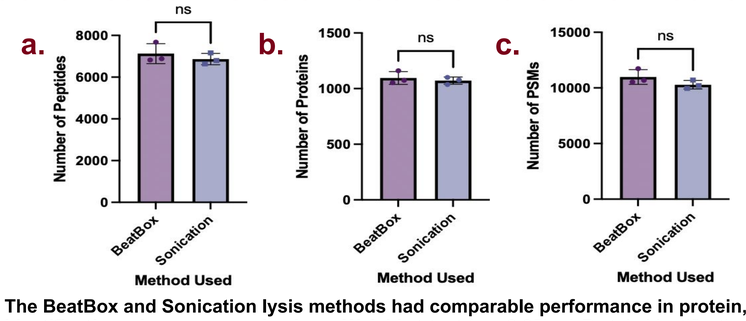

Figure 4: The BeatBox and Sonication lysis methods had comparable performance in protein, peptide, and PSM identification when compared using a Welch's t-test ($p < 0.05$). a) Protein identification was not significantly different between the two methods. In the BeatBox 1096 ± 53 proteins were identified, while in Sonication 1072 ± 30 proteins were identified ($p= 0.5685$). **b)** Peptide identification was not different between the two methods. In the BeatBox 7128 ± 431 peptides were identified, while in Sonication 6866 ± 248 were identified ($p= 0.4653$). **c)** PSM identification was not significantly different between the two methods. In the BeatBox 10981 ± 598 PSMs were identified, while in Sonication 10287 ± 359 PSMs were identified ($p= 0.2074$).

Conclusions and Future Directions

Conclusions:

- BeatBox performs equivalently to the sonication methods for protein, peptide, and PSM identification.
- However, the BeatBox does outperform the sonication method in total protein quantification.
- The lysis process may be more efficient in the BeatBox, but not enough to impact identification.

Future Directions:

- Assess the biochemical properties of the proteins with differential relative abundances found in the BeatBox vs. the Sonication method.