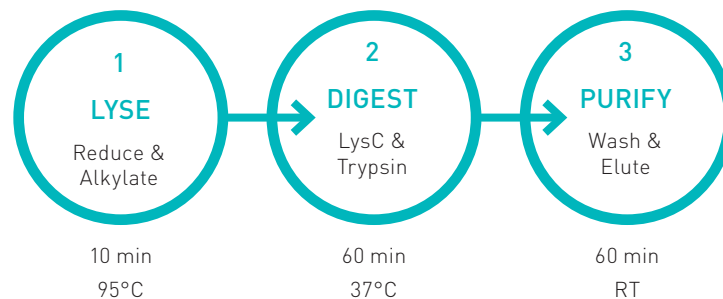# iST Sample Preparation with the Bioruptor®

Objective

The PreOmics iST sample preparation of proteins combined with the Diagenode Bioruptor® Pico enhances mass spectrometric analyses.

1 LYSE Reduce & Alkylate — 10 min 95°C → **2 DIGEST** LysC & Trypsin — 60 min 37°C → **3 PURIFY** Wash & Elute — 60 min RT

Background

Mass spectrometry (MS)-based proteomics typically employs multiple sample processing steps, representing a crucial part of routine MS analyses (1). Complex workflows, extensive sample fractionation and proteolytic digestion are highly time-consuming and restrict the overall technical reproducibility. The accuracy and robustness of the MS platform is also strongly affected by sample quality reasoning for high quality proteomic samples. In this application note, we demonstrate that the straightforward and robust in-StageTip (iST) Kit in combination with sample sonication by the Bioruptor® Pico (2) outperforms sample preparation workflows that do not use sonication or that use only heat treatment.

Material and methods

Baker`s yeast (*Saccharomyces cerevisiae*) derived from a yeast cube was resuspended in PBS. Aliquots of OD_{600} = 1 were harvested and centrifuged. Pellets were frozen at -20 °C until use. Each cell pellet was then resuspended in 50 µl LYSE and the following conditions were tested:

Condition	Boiling at 95 °C (min)	Bioruptor® Pico sonication (cycle time)*
1	0	0
2	10	0
3	0	10
4	10	1
5	10	2
6	10	5
7	10	10

*Each cycle corresponds to 30 sec ON and 30 sec OFF

After the heat and/or sonication treatment, each sample was transferred to the CARTRIDGES, before continuing with the protocol according to the iST Sample Preparation Kit instructions. MS analysis was performed basically as described (1) on a QE Exactive mass spectrometer (Thermo Fisher Scientific). Data analysis was performed using the MaxQuant (2) and Perseus software tools (3). Biological triplicates were used throughout the experiment.

Results

We examined several parameters that indicate efficient reduction, alkylation and digestion of the protein samples, such as number of peptide and protein identifications or alkylation rates. When comparing the seven distinct experimental conditions, the number of peptide and protein identification correlated with the number of sonication cycles, increasing protein and peptide identifications by up to 22% and 72%, respectively:

Condition	#proteins	#peptides	Alkylation [%]	protein ID change relative to condition 1	peptide ID change relative to condition 1
1	1721	4933	1.0	-	-
2	1671	4652	1.4	-3%	-6%
3	1856	7007	7.4	+8%	+42%
4	1918	7446	7.5	+11%	+51%
5	1936	7584	7.6	+13%	+54%
6	1984	7867	8.2	+15%	+59%
7	2093	8476	7.9	+22%	+72%

Principle component analysis (PCA) revealed clear separation of the samples prepared with or without the Bioruptor (Figure 1A+B). Upon closer inspection, we found that many proteins were only identified when the yeast cells were lysed by heat and sonication (Figure 1C+D). Pathway enrichment analysis of GeneOntology annotation terms revealed a significant enrichment of terms such as "fungal cell type wall" (GOCC, enrichment factor >6.2, p<6.2E-06, Benjamini-Hochberg controlled false discovery rate), "cytoplasmic stress granules" (GOBP, EF>2.5, p<4.5E-06 BH-FDR) or "mitochondrion" (GOCC, EF>1.9, p<3.1E-06 BH-FDR) among the Bioruptor-specific identifications.
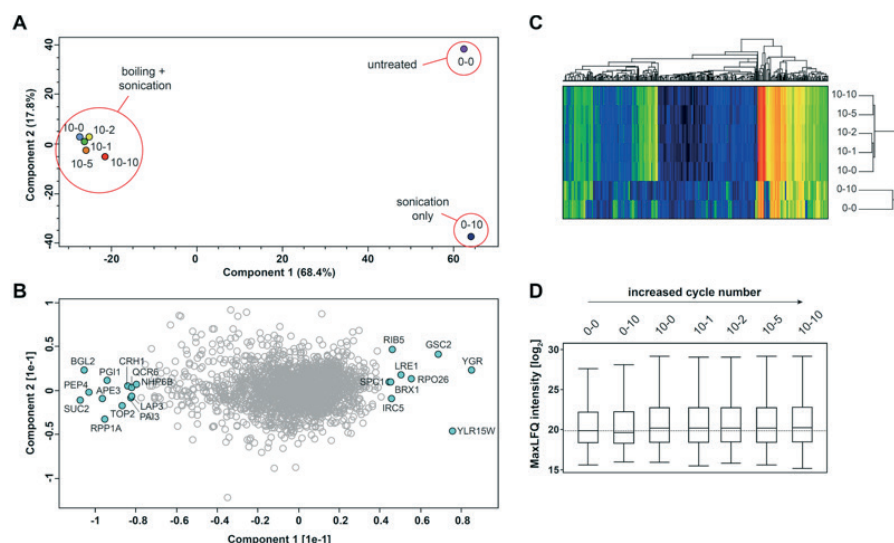
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Figure 1

A. Principle component analysis, projections. Triplicate sample preparation and MS analysis show very good separation of Bioruptor-treated or non-treated samples.

B. Principle component analysis, loadings. The proteins responsible for the separation of the three sample preparation groups are depicted in Fig. 1B. Exemplified proteins driving the separation are marked in cyan.

C. Unsupervised hierarchical clustering highlights clusters of proteins that are present in Bioruptor-treated and absent in non-treated samples.

D. Boxplot of MaxLFQ protein intensities (log2FC) demonstrates higher protein intensities for increasing cycles of Bioruptor treatment compared to non-treated samples (median indicated by dashed line).

Conclusion

We show that sample preparation with the iST Kit combined with the Bioruptor® Pico outperforms the workflow with the heat treatment alone. Thus, the sonication-based cell lysis aids in proteome-wide discovery by greatly enhancing the number of peptide and protein identifications.

References

1. NA Kulak, G Pichler, I Paron, Nagaraj N and M Mann. *Minimalistic, encapsulated proteomic-sample processing applied to copy number estimation in eukaryotic cells.* Nat Methods (2014) 11(3):319-24. doi: 10.1038/nmeth.2834.

2. S Tyanova, T Temu and J Cox. *The MaxQuant computational platform for mass spectrometry-based shotgun proteomics.* Nat Protocols (2016) 11(12):2301-19. doi: 10.1038/nprot.2016.136

3. S Tyanova, T Temu, P Sinitcyn, A Carlson, MY Hein, T Geiger, M Mann and J Cox. T*he Perseus computational platform for comprehensive analysis of (prote)omics data.* Nat Methods (2016) 13:731-740. doi:10.1038/nmeth.3901.

Ordering information

Product	Quantity	Manufacturer	Code
iST Sample Preparation Kit	8 reactions	PreOmics GmbH	P.O.00001
	96 reactions		P.O.00027
Bioruptor® Pico	1 unit	Diagenode SA	B01060001

For further information, please see our websites:

http://www.preomics.com

https://www.diagenode.com

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