BIORUPTOR® PROTOCOL

MASS SPECTROMETRY – USING THE THE BIORUPTOR® TO LYSE C.ELEGANS FOR BIOCHEMICAL AND PROTEOMIC ANALYSIS

Prasad Kasturi and F Ulrich Hartl from the Department of Cellular Biochemistry at the Max Planck Institute for Biochemistry, Martinsried, Germany are using the Bioruptor® for their research using *C. elegans*, a powerful metazoan model system to study and understand fundamental problems in biology. Traditionally *C. elegans* research has focused mainly on genetics. However, recent studies suggest that *C. elegans* can also be a facile system for biochemistry. Tagged proteins can be expressed *in vivo* and interacting partners can be identified using various biochemical approaches. In addition, protein networks and pathways can be identified by combining genetic screens with biochemistry.

Synchronized worm populations are easy to grow in large quantities to isolate protein amounts sufficient for biochemical analysis. However, the specific method used for worm disruption lysis may affect protein yield and total number of identifiable proteins, and may also influence downstream processing, such as mass spectrometric analysis and co-immunoprecipitation. Thus, protein extraction procedures must be carefully optimized when analysing protein-protein interactions and performing proteome wide quantifications.

Figure 1. *C.elegans worms at different stage of their life.*

Methods of choice for worm lysis

Various methods are available for worm lysis. These include sonication, grinding and the use of a bead beater. Each of these methods has their advantages and disadvantages.

Sonication

Freeze/thaw cycles help to make the worms fragile and break open the cuticle. Sonication of these worms helps to lyse them efficiently. Sonication is performed by using a microtip that is inserted into the sample. A Dounce homogenizer may facilitate the lysis process. In both of these methods the metal tip is in direct contact with the sample and may cause undesired heating. If there are multiple samples, then the metal tip needs to be cleaned after each round of sonication. The overall process can be lengthy and the efficiency of lysis may vary among the samples.

Grinding

Worms are lysed by grinding in liquid nitrogen, followed by sonication. A cold mortar and pestle are used to disrupt frozen worm pellets in liquid nitrogen. The resulting powder is suspended in a suitable buffer, followed by sonication. A large number of worms are required. The process can be tedious and time consuming if there are multiple samples.

Bead beater

The bead beater can disrupt worms quickly, however it has a set volume of 1.5 - 2 ml that must be used. Moreover, temperature fluctuations during bead beating result in highly variable protein recoveries and also cause protein denaturation. Loss of integrity of protein complexes may occur.

Procedure worm lysis with the Bioruptor®

The bioruptor® is a water bath sonicator in which the lysis of samples occurs without inserting any kind of microtip. A controlled cooling system maintains the temperature. Up to 12 samples of any volume can be lysed at the same time. All samples are treated in the same way, saving time and ensuring reproducibly high protein yields.

The instrument is particularly useful when different samples need to be lysed in replicates. The lysis procedure allows a reliable quantitative comparison for different samples and maintains the integrity of protein complexes.

1. **Buffer preparation**

M9 buffer		*Lysis Buffer (for co-IPs)*		*Lysis Buffer (for isolation of insoluble proteins)*	
22 mM	KH_2PO_4	10 mM	Tris HCl (pH 7.5)	50 mM	Tris HCl (pH 8.0)
42.25 mM	Na_2HPO_4	150 mM	NaCl	0.5 mM	NaCl
85.56 mM	NaCl	0.5 mM	EDTA	4 mM	EDTA
		0.5 %	NP-40	1 % (v/v)	NP-40
		Complete proteinase inhibitor cocktail		Complete proteinase inhibitor cocktail	

2. **Procedure**

1. Wash the worm plates with M9 buffer and collect them into 1.5 ml Eppendorf Safe-Lock tubes.

   Comment: In each tube 100 to 1000 adult worms can be collected.

2. Briefly spin at 1000g for 1 minute. Wash one more time with M9 buffer and store the worm pellets at -80°C until use.

3. Before lysing the worms, add appropriate lysis buffer to the worm pellets.

   Comment: Lysis buffer volume depends on the numbers of worms and influences the protein concentration. 300µl to 400 µl of lysis buffer is required to lyse 500 adult worms.

4. Lyse the worms in Bioruptor® at high power for 10 cycles (sonication cycle: 30 sec ON, 30 sec OFF). For aged old worms add 5 to 10 more cycles.

5. Clarify the lysates by spinning at 1000g for 1 min. Transfer the clarified lysates to new tubes and measure the protein concentrations.

Usage and Reproducibility

Prasad Kasturi and F Ulrich Hartl used the bioruptor® to lyse wild-type worms as well as different lifespan mutant worms of different ages in replicates. Lysates of these worms were used to identify proteome wide changes that occur during aging by SILAC quantitative proteomics. Their results suggested that proteomic analyses are highly reproducible within biological and technical replicates, indicating highly reproducible protein yields.

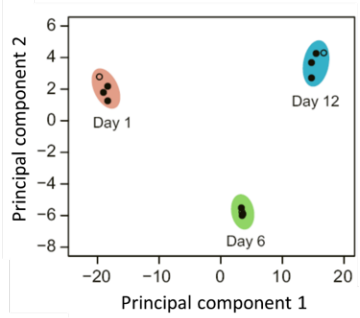

Figure 2. *Reproducibility of proteome analysis in multiple replicates of worm samples from different ages (adopted from Walther DM and Kasturi P et al., Cell. 2015).*

Author's conclusion

"We have compared different lysis methods to prepare lysates from C.elegans strains. We obtained the most reliable and reproducible results with the bioruptor. Advantages of the bioruptor are that multiple samples can be lysed simultaneously, cross-contamination of samples is avoided and temperature can be well controlled. Bioruptor is the choice of method for worm lysis when performing accurate protein quantifications and co-immunoprecipitation experiments."

Publications

1. Walther DM, Kasturi P, Zheng M, Pinkert S2 Vecchi G, Ciryam P, Morimoto RI, Dobson CM, Vendruscolo M, Mann M, Hartl FU. **Widespread Proteome Remodeling and Aggregation in Aging C. elegans.** *Cell. 2015 May 7;161(4):919-32. doi: 10.1016/j.cell.2015.03.032.*

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