ApplicationForum

FastPrep® Systems for Sample Preparation for Proteomic Characterization with Mass Spec

Jeffrey D. Whyte, Global Product Manager *MP Biomedicals Inc.*, 3 Hutton Centre Drive, Santa Ana, CA USA 92707

Introduction

Sample preparation techniques for Mass Spectrometry (MS) vary widely, due to the complexity of the proteome. Sample type, biological questions to be answered and instrumentation all have influence on sample prep protocols, and factors such as reproducibility, hands-on time and cost also need to be considered. The FastPrep Family of Sample Preparation Instruments and Kits satisfy these rigorous demands. **(Fig. 1).** FastPrep are benchtop instruments based on bead-beating technology. They provide complete and quantitative lysis of challenging and routine samples and are suitable in all applications that require grinding, lysing or homogenization. Mechanical lysis offers rapid cell disruption for the isolation of DNA, RNA, proteins, metabolites and other small molecules, eliminating the need for chemicals, enzymes and detergents, which can potentially inhibit downstream processes. Samples include but are not limited to: all types of human, animal and plant tissues, including cultured cells; bacterial, yeast and fungal cells, including spores; and environmental samples, including soil and feces. The FastPrep-24™ 5G instrument employs a unique, optimized motion to disrupt cells through the multidirectional, simultaneous beating of specialized Lysing Matrix beads on the sample material. FastPrep-24 5G is the only homogenizer with eleven optional adaptors allowing for unique processing versatility, with tube sizes of 2, 4.5, 15 and 50 mL available for processing samples under ambient or cryogenic temperature conditions. A wide range of Lysing Matrix tubes are available and contain beads of varying materials, sizes and shapes, optimized by sample type and guarantee thorough homogenization every time.

Figure 1. FastPrep Instruments and Lysing Matrix Tubes, Simplify your workflow.

Examples of FastPrep in MS workflows

Photosynthetic reaction centers found in bacteria, plants and algae contain the chlorophyll-protein complexes that convert light energy into stable charges in biological organisms and are of interest in the development of renewable energy sources. Photosystem I (PSI) is one reaction center that is found in its monomeric form in plants/algae and as trimers in most cyanobacteria. Li *et al* [1] discovered a predominantly tetrameric form of PSI in the thermophilic cyanobacteria *Chroococcidiopsis* sp TS-821 (TS-821). In this study cultured TS-821 cells were pelleted and their thylakoid membranes isolated using a FastPrep-24 instrument and lysing matrix tubes with a buffer consisting of 50 mM MES-NaOH, pH 6.5, 5 mM CaCl_2 , 10 mM MgCl_2 with 25% glycerol. The resulting homogenate was centrifuged at 180,000 x g for 1 hour to pellet the thylakoid membranes. To isolate PSI the thylakoid membranes were solubilized in varying concentrations of dodecyl-maltoside (DMM) buffer and analyzed on Blue Native PAGE gradient gels, 4-10%. As a control, identical analysis was done on the well-characterized PSI from *Thermosynechococcus elongates*. DMM concentration dependent BN-PAGE bands confirmed predominantly tetrameric PSI in TS-821 (with some monomer and dimer) versus the PSI trimeric bands and PSII dimeric and monomeric bands from *T. elongates*. They then excised the PSI green bands from TS-821 and ran a second dimension 10% SDS-PAGE gel to separate the PSI subunits as detected by silver staining. To further isolate tetrameric PSI from TS-821, thylakoid membranes were solubilized and subjected to a two-step Sucrose Density Gradient fractionation, 10-30% with dialysis concentration between centrifugations. The resulting fractions were also resolved into subunits by SDS-PAGE. Subunit identities were confirmed by LC MS/MS peptide analysis. The subunit bands were excised, destained with 50 mM NH_4HCO_3 pH 8.0 and dehydrated. Bands were rehydrated and treated with buffers to reduce disulfide bonds and to alkylate cysteines, prior to trypsin digestion. TS-821 tetramer protein was denatured in 7 M urea with 8.3 mM DTT and then diluted in 50 mM Tris and 10 mM CaCl_2 prior to treatment with Trypsin Gold. Samples were loaded onto an LTQ-Velos MS for peptide extraction, quantification and fragmentation. The resulting tandem mass spectra was compared to putative proteomic databases for confirmation and evolutionary phylogenetic mapping of TS-821. This complete characterization of the tetrameric form of PSI in TS-821 also shows it an attractive candidate for bio-derived solar energy production.

Comparing proteins of interest to isotope-labelled standards is a powerful technique for quantitative analysis MS-based studies. However, these standards are expensive and limit study flexibility as they must be commercially available or synthesized, which is also time consuming. Therefore, label-free protocols are becoming popular, but due to the lack of an internal reference, more precise sample preparation is necessary. Vowinckel *et al* [2] compared sample prep methods based on three popular digestion formats; *in gel*, *filter aided* and *in solution*. Resulting label-free samples were analyzed, on a 5600 QqTOF LC MS/MS instrument, and results compared for sample prep complexity, precision in quantitative analysis and cost. All samples started from a single culture of *Saccharomyces cerevisiae*, aliquoted to 10 OD₆₀₀ units

and pelleted to 30 mg, and all final peptide samples were in 50 μ L of 5% acetonitrile(ACN)/0.1% formic acid. Two *in gel* protocols were benchmarked, comparing protein extraction lysis buffers. One utilized an SDT buffer of 4% SDS, 100 mM Tris/HCl pH 7.6, 0.1 M DTT, and the other was SDS-free, 0.5M ammonium bicarbonate (ABC.) For both, 200 $\pm$ 10 mg glass beads and 200 μ L lysis buffer were added to the yeast pellet. Cell disruption was by FastPrep-24 at a speed setting of 6.5 M/sec for 3 cycles of 20 sec with 5 minute ice incubations in between. Lysates were centrifuged at 16,000 x g for 5 minutes, supernatants transferred to a clean tube, then centrifuged again. Cleared supernatant was assayed by BCA, and proteins separated by SDS-PAGE. Bands were excised and dehydrated with ABC and ACN, reduced with DTT, alkylated with iodoacetamide, then trypsin digested, all while in the gel. *Filter aided* protocols utilize protein concentration centrifuge filter units with digestion taking place on the filter. Cell lysis was by FastPrep-24 as described above, with the cleared lysate then spun through the filter, capturing proteins. Urea was applied to the filter, followed by Lys-C/trypsin for digestion. Peptides were desalted on an SPE Extraction Cartridge C18 with several washes of trifluoroacetic acid. The *in solution* protocol used identical FastPrep cell disruption. Cleared lysates were diluted with ABC, treated with ACN and then a buffer of 45 mM DTT/20 mM tris(2-carboxyethyl)phosphine, followed by iodoacetamide alkylation and trypsin digestion, all performed in solution. The final sample was cleaned with the same filter apparatus used in the filter aided method. While all the methods have value in certain experimental conditions, the *in solution* method gave the best results for label-free proteomic quantitative analysis by LC MS/MS.

Dieme *et al* [3] developed a proteomic method for mosquito species identification at the aquatic larval stage of the life cycle utilizing MALDI-TOF-MS. Vector control measures have advantages when aimed at aquatic breeding sites. Larvae are nonbiting, thus not active transmitters, and are confined to the breeding site, making eradication easier and more effective. Because multiple species can be present, taxonomic identification and population densities are critical to direct treatments. However, larval morphological-based identification is problematic. Entomological expertise is necessary, laborious and prone to error. PCR and other molecular methods analyze multiple genes to differentiate species, resulting in more sequencing, adding time and cost to validate field efforts. This proteomic method was developed as a rapid, economical alternative, and was optimized for variables including sample preparation and sample storage conditions. Each whole larva was homogenized in 70% HCOOH by two methods: manual grinding using a micro-pestle, and FastPrep-24 processing in the presence of glass beads. The resulting homogenates were mixed with 50% ACN and centrifuged for clearance. One μ L of supernatant was deposited onto the steel target plate, and 1 μ L of CHCA was directly overlaid and dried, after which the plate was loaded into the MALDI-TOF-MS instrument. The resulting protein spectra profiles created a database that correctly distinguished individuals from the *Anopheles*, *Aedes* and *Culex* genres. FastPrep sample preparation yielded MS profiles (**Fig. 2**) with quality equal to that of the manually prepared samples, leading the authors to recommend automated sample prep for high-throughput workflows.

Figure 2. Effect of sample preparation methods and storage conditions on spectra quality. Comparison of the MALDI-TOF-MS spectra of whole *An. gambiae* at the L3 stage homogenized manually with a pestle (a); homogenized automatically with a FastPrep apparatus (b); homogenized manually in deionized water and then subjected to acidic extraction (c); stored at -20°C for 60 d and homogenized manually with a pestle (d); and stored in 70% ethanol for 7 (e), 14 (f) and 60 (g) d. a.u., arbitrary units; m/z, mass-to-charge ratio. Adapted with permission from ref. 3.

Conclusions

The FastPrep Family is an ultra-high-performance sample preparation system that allows for the extraction of fully intact, biologically functional macromolecules from routine and highly resistant samples and is well suited for MS-based proteomic applications. MP Biomedicals offers solutions for other research applications from low- to high-throughput scale with a selection of sample preparation instruments, Lysing Matrix tubes and complete purification kits. Learn more at <http://www.mpbio.com/sampleprep>.

References

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