Application Note

Genomics

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Empowering High-Throughput Proteomics Workflows with the Agilent ProteoAnalyzer System

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Abstract

This application note shows how the Agilent ProteoAnalyzer capillary electrophoresis system is used at Majorbio, a high-throughput proteomics laboratory, for automated protein sample quality control (QC). Protein sample QC is a critical step in the proteomics workflow, providing a reliable foundation for obtaining high-quality omics data. Traditional methods such as SDS-PAGE are often cumbersome, labor-intensive, time-consuming, low in sensitivity, and difficult to integrate with automated data management. These limitations have become bottlenecks in scaling throughput and automation in modern proteomics laboratories.

The ProteoAnalyzer addresses these challenges through automation, high throughput, and sensitivity. Majorbio's capacity for protein sample QC is enhanced with the implementation of the ProteoAnalyzer in their workflow by reducing manual intervention and enabling digital and automated management of analytical results. The system also supports effective analysis of low-concentration samples and provides automated quantification, further streamlining workflows and significantly improving operational efficiency.

Introduction

Proteomics research plays a vital role in uncovering complex biological processes and elucidating disease mechanisms. Currently, mass spectrometry-based bottom-up proteomics analysis is the most widely used approach. This workflow typically includes protein extraction, quality control, enzymatic digestion, desalting, chromatographic separation, mass spectrometry analysis, and data interpretation (Figure 1). In high-throughput proteomics laboratories, automation of each step and the elimination of throughput bottlenecks are essential for achieving efficient, high-quality, and cost-effective operations.

Compared with traditional SDS-PAGE, the ProteoAnalyzer offers significant advantages in sample throughput, data quality, and workflow simplification. The ProteoAnalyzer delivers high sensitivity and reproducibility. For example, using BSA dissolved in PBS, the detection limit is as low as 1 ng/µL[1], enabling reliable analysis of low-concentration samples.

For analysis with the ProteoAnalyzer, each sample is prepared with internal markers: a lower marker (LM, 6 kDa) and an optional upper marker (UM, 240 kDa). These markers allow for alignment of the samples with a known ladder, providing reproducible and reliable sizing and quantification data.[2]

In contrast, SDS-PAGE is limited in both sensitivity and accuracy. For example, when the sample loading is too high, sizing data may be inaccurate; when the loading is too low, bands may be undetected due to low sensitivity. Further, SDS-PAGE has a narrow linear concentration range, typically spanning only two orders of magnitude or less, leading to imprecise quantification when the sample concentration is too high or too low. The ProteoAnalyzer overcomes this limitation by offering a broader linear quantification range of three orders of magnitude, accommodating a wide range of sample concentrations.[3]

Additionally, the ProteoAnalyzer improves the operational efficiency of the laboratory by increasing throughput and reducing manual workloads. As shown in Figure 2,

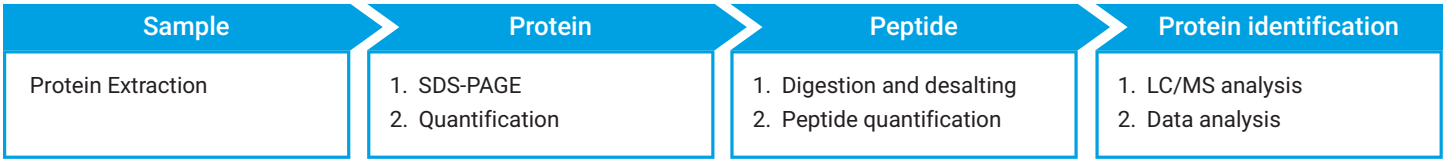

Figure 1. Bottom-up proteomics workflow starting from sample extraction, followed by protein quantification using SDS-PAGE, peptide digestion and desalting, and concluding with LC/MS analysis for protein identification.

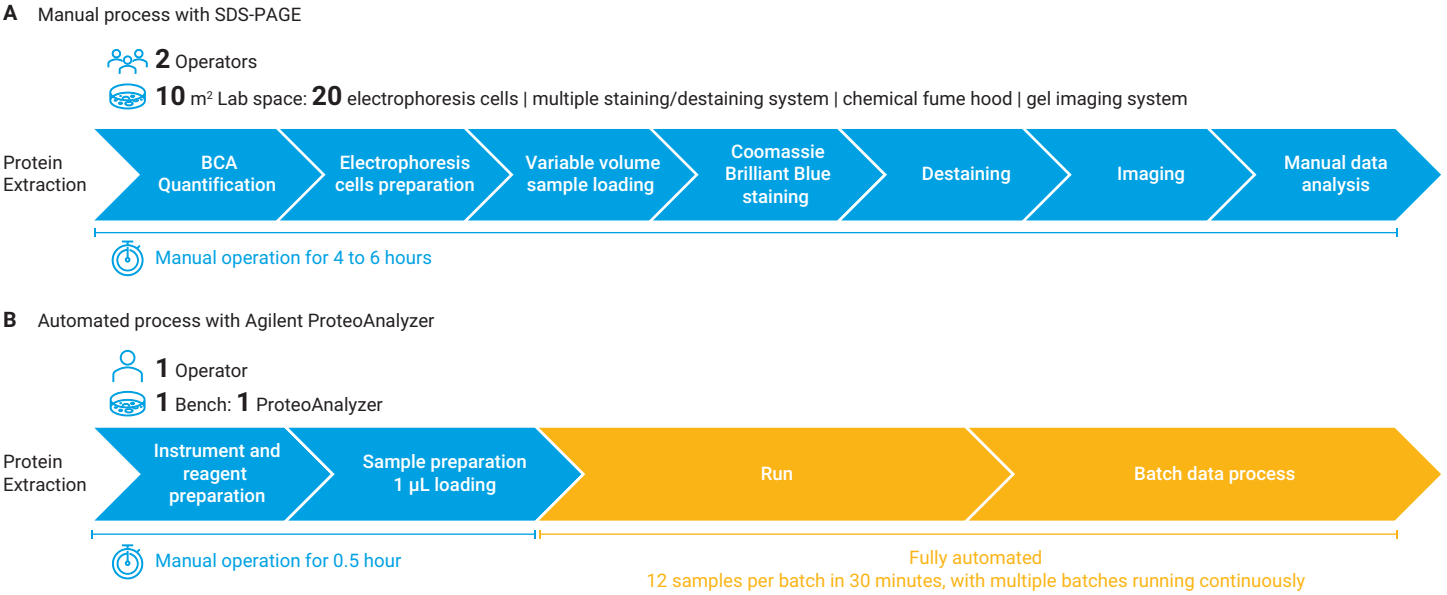

Figure 2. The Agilent ProteoAnalyzer enhances the operational efficiency of high-throughput laboratories. (A) The manual workflow using SDS-PAGE; (B) the streamlined, automated process enabled by the ProteoAnalyzer, which enhances operational efficiency in high-throughput laboratory settings.

a high-throughput proteomics laboratory processing 200 samples via SDS-PAGE requires at least two technicians, 4 to 6 hours of manual operation, approximately 20 electrophoresis cells, and staining/destaining steps that must be performed in a fume hood. Alternately, the ProteoAnalyzer enables a single technician to prepare samples in just 30 minutes. The system can automatically process multiple batches, analyzing 12 samples every 30 minutes, significantly accelerating the workflow from sample preparation to result generation.

In proteomics laboratories, the ProteoAnalyzer frees users from the labor-intensive SDS-PAGE workflow and eliminates the need for pre-measuring sample concentrations due to its high sensitivity and wide linear concentration range. Equal-volume loading can be performed directly, avoiding the need to adjust loading volumes due to concentration differences, thereby improving overall lab efficiency. Additionally, the ProteoAnalyzer has a small footprint, conserving bench space and eliminating the need for a fume hood and imaging equipment, resulting in a cleaner, more organized, and more efficient laboratory environment.

Experimental

Sample information and protein extraction

Different types of samples require different protein extraction methods based on their characteristics. The protein extraction method used for animal tissues is described here as an example. Frozen samples are transferred to grinding tubes and an appropriate amount of protein lysis buffer containing protease inhibitors is added. Next, the samples are shaken three times using a cryogenic grinder for 180 seconds each time. This is followed by 30 minutes of non-contact ultrasonication, then centrifugation at 14,000 g for 15 minutes at 4 °C. The supernatant is collected as the extracted protein.

Protein quality control

After protein extraction, the samples were analyzed with both the ProteoAnalyzer and SDS-PAGE for QC. The workflows of both methods are shown in Figure 3.

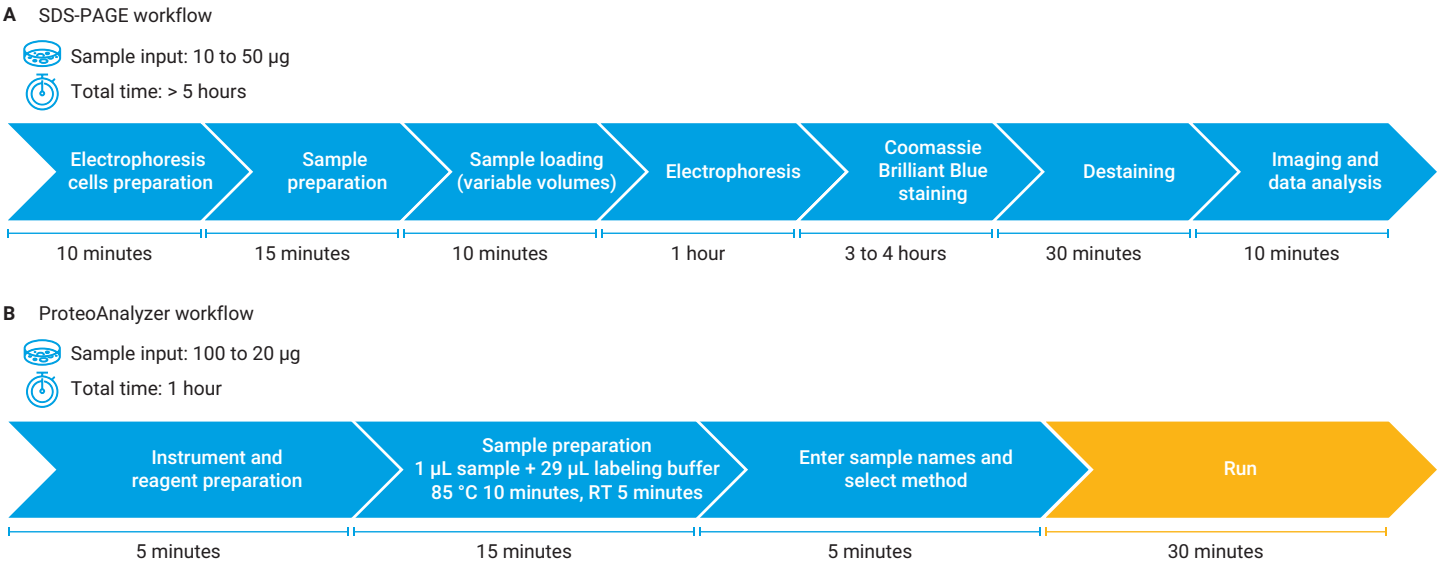

Figure 3. Comparison of SDS-PAGE and Agilent ProteoAnalyzer workflows. The SDS-PAGE process involves multiple manual steps including electrophoresis, staining, and destaining, requiring several hours of hands-on operation. In contrast, the ProteoAnalyzer workflow streamlines protein analysis with a consistent sample input volume and minimal manual intervention.

SDS-PAGE quality control method

Due to the low sensitivity and narrow concentration range of SDS-PAGE, it is necessary to quantify the samples using the bicinchoninic acid (BCA) method and adjust the loading volume accordingly before electrophoresis. Based on the quantification results, 10 µg of sample is mixed with loading buffer containing DTT, heated at 95 °C for 5 minutes, and manually loaded into a precast SDS-PAGE gel. Electrophoresis is performed at 180 V for 40 to 50 minutes. After electrophoresis, the gel is removed and stained/destained with Coomassie Brilliant Blue (for 4 hours to overnight). Finally, the gel is imaged using a gel imaging system, with manual parameter adjustment, sample annotation, and report editing.

ProteoAnalyzer quality control method

Since the ProteoAnalyzer is compatible with both high- and low-concentration protein samples (providing linear quantification across three orders of magnitude)[2], there is no need for additional BCA quantification or sample volume adjustment prior to analysis. Using the ProteoAnalyzer, all samples obtained from the above protein extraction method can be loaded directly with a volume of 1 µL.

Samples were analyzed using the Agilent Protein Broad Range P240 kit under reducing conditions per the manufacturer's instructions. Briefly, using the Protein Broad Range 240 kit under reducing conditions, 1 µL of extracted protein is mixed with 29 µL of the prepared labeling master mix. The samples are then heated at 85 °C for 10 minutes, incubated at room temperature for 5 minutes, centrifuged, and loaded onto the ProteoAnalyzer for automatic analysis. The Agilent ProSize data analysis software can automatically generate data reports and can be integrated into automated workflows for data extraction and report generation.

Enzymatic digestion, peptide quantification, and LC/MS analysis

Total protein (50 µg) is used as initial starting material. Reduction and alkylation are performed, then trypsin is added for enzymatic digestion. The digested product is desalted, dried using a vacuum concentrator, reconstituted in 0.1% formic acid, and quantified using a NanoDrop. Based on the peptide quantification results, the sample is diluted to a concentration of 100 ng/µL, and 1 to 3 µL is used for LC/MS analysis. After mass spectrometry acquisition, data analysis software is used for database searching and interpretation.

Results and discussion

ProteoAnalyzer and SDS-PAGE comparison

To evaluate performance, human cell line samples were analyzed with both SDS-PAGE and on the ProteoAnalyzer. For SDS-PAGE, 10 µg of protein per sample based on BCA quantification was loaded into each well. In parallel, the same samples were analyzed using the ProteoAnalyzer by directly loading 1 µL of each sample. Figure 4 shows the SDS-PAGE gel image (A) and the digital gel image from the ProteoAnalyzer (B). The band distributions observed in the SDS-PAGE and ProteoAnalyzer digital gels were highly similar. The inclusion of LM and UM internal markers in the ProteoAnalyzer ensures high reproducibility in band distribution. In addition, the ProteoAnalyzer generates an electropherogram for each sample, displayed in Figure 4C. An overlay of the electropherograms allows for more intuitive analysis of sample reproducibility and differences (Figure 4D).

Stability and compatibility of the ProteoAnalyzer for protein detection across diverse sample types

Majorbio has implemented the ProteoAnalyzer for protein QC across a wide range of sample types, including cell lines, animal tissues, plant tissues, fungi, bacteria, and algae. High-quality QC data have been obtained for proteins from each of these diverse sources, demonstrating the strong compatibility of the ProteoAnalyzer with complex sample types (Figure 5).

Advantages of the ProteoAnalyzer in large-cohort protein sample analysis

The ProteoAnalyzer offers unique advantages for large-cohort sample analysis. The instrument can perform rapid, automated, and continuous analysis of batch samples, improving detection efficiency. The inclusion of LM and UM markers ensures stability and consistency of sample results. The integrated Agilent ProSize data analysis software enables direct comparison of samples analyzed across different batches and time points, making it easy to visualize and compare QC results for large sample sets.

Figure 6 shows the digital gel images of 92 mouse liver tissue samples and 90 adipose tissue samples analyzed using the ProteoAnalyzer. Following protein extraction, all samples were loaded at a uniform volume of 1 µL for ProteoAnalyzer analysis. Data was collected over nine runs, and the ProSize software was used to compile and analyze all digital gel images. This approach allows for rapid assessment of sample quality and evaluation of consistency or differences across the cohort.

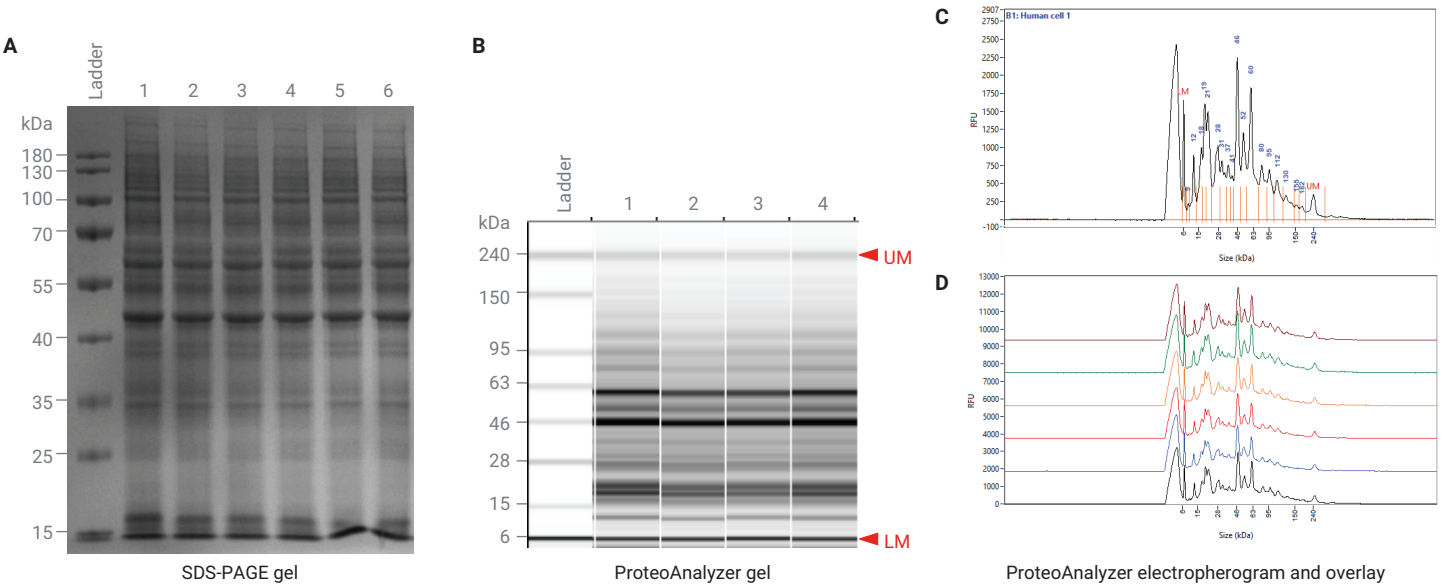

Figure 4. Comparison of Agilent ProteoAnalyzer and SDS-PAGE results for human cell line samples. (A) SDS-PAGE gel image. (B) ProteoAnalyzer digital gel image. (C) ProteoAnalyzer electropherogram of sample 1. (D) ProteoAnalyzer electropherogram overlay of samples 1 to 6.

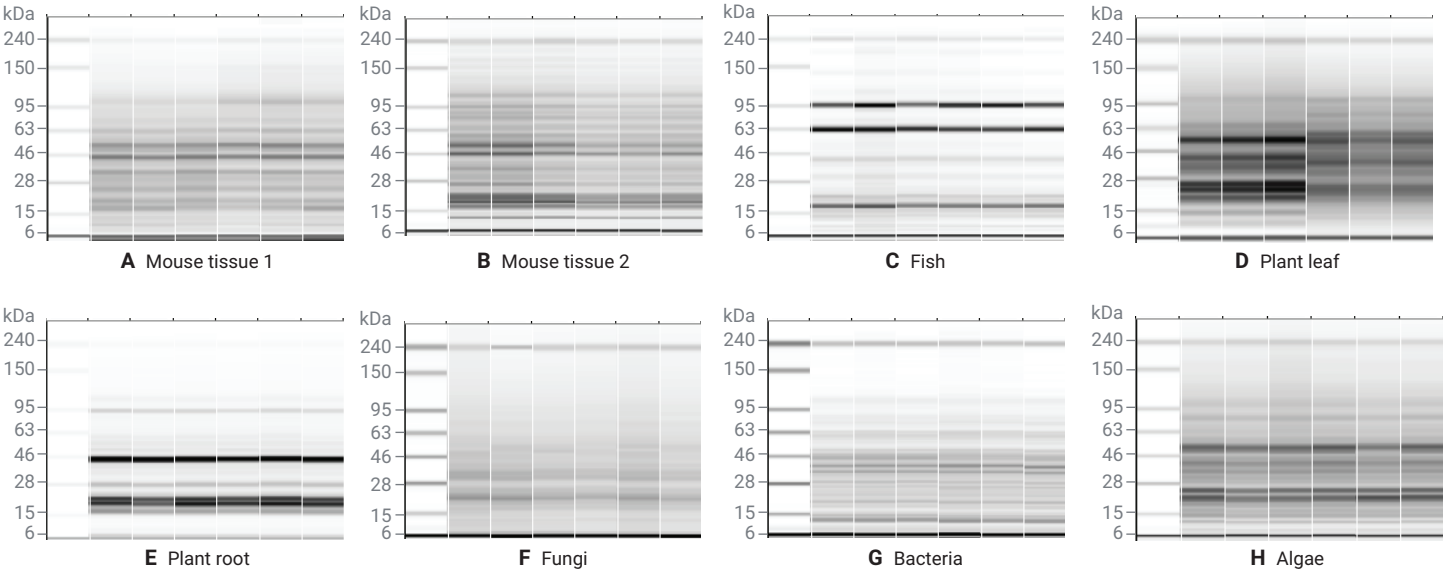

Figure 5. Digital gel images generated by Agilent ProteoAnalyzer. Samples were derived from different species, including: (A, B, and C) animals, (D and E) plants, (F) fungi, (G) bacteria, and (H) microalgae. Each digital gel image contains six samples and a ladder.

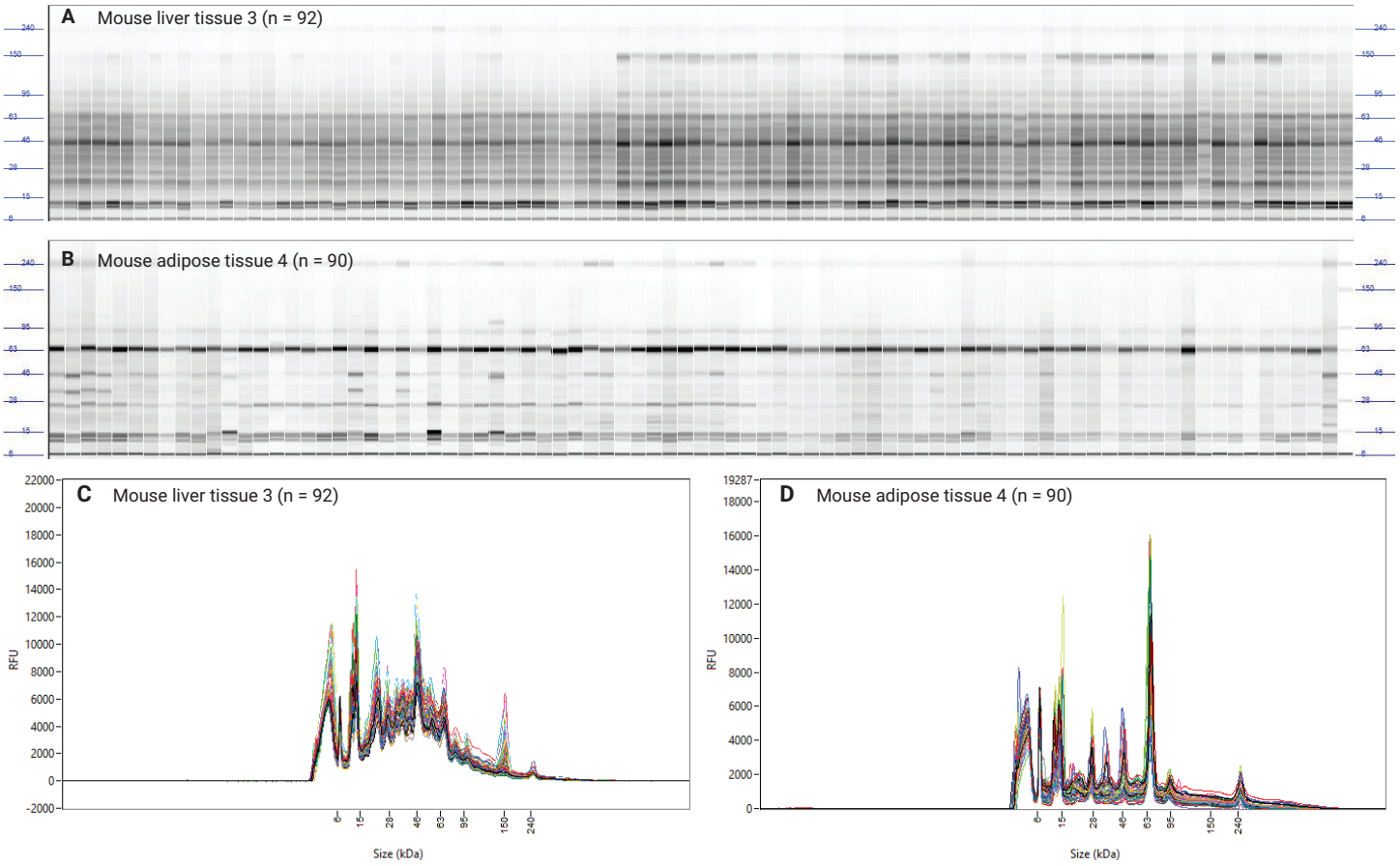

Figure 6. Digital gel image and electropherogram overlay of large-cohort samples generated by the Agilent ProteoAnalyzer. (A and C) Digital gel image and electropherogram overlay of mouse liver tissue 3 (n = 92). (B and D) Digital gel images and electropherogram overlay of mouse adipose tissue 4 (n = 90).

Protein quantification in the proteomics workflow using the ProteoAnalyzer

From extensive sampling assessment based on data from a large number of samples, Majorbio has identified a streamlined workflow for their QC analysis. First, the sample is quantified using the ProteoAnalyzer system. A total protein amount of 30 µg as determined by the ProteoAnalyzer has been deemed sufficient for downstream proteomics analysis. Thus, 30 µg of the total protein is then used to perform enzymatic digestion and desalting to obtain peptides. The peptide concentration is measured using a peptide quantification kit and normalized to 100 ng/µL. Finally, 3 µL from each sample (a total of 300 ng of peptide) is analyzed on a high-resolution mass spectrometer with an 8-minute acquisition time. Table 1 summarizes the experimental results of five different sample types. All samples yielded a high number of protein identifications, demonstrating the reliability of the ProteoAnalyzer quantification capabilities in the proteomics workflow.

Table 1. Number of proteins identified by high-resolution mass spectrometry across five sample types (n indicates the number of replicates per sample type). Protein counts represent the total number of unique proteins detected across all replicates.

Sample Types and Counts	Number of Proteins Identified by High-Resolution Mass Spectrometry
Mouse Tumor Cells (n = 10)	9,683
Human Cells (n = 12)	8,723
Mouse Cells (n = 6)	8,543
Rat Brain Tissue (n = 9)	7,953
Mouse Lung Tissue (n = 36)	10,628

Conclusion

The Agilent ProteoAnalyzer system, with its high sensitivity, automation, and throughput, has significantly enhanced the operational efficiency and data quality of high-throughput proteomics laboratories. Compared with traditional SDS-PAGE methods, the ProteoAnalyzer simplifies workflows, reduces labor costs and time requirements, and improves the accuracy and consistency of protein QC. The broad concentration range eliminates the need for pre-adjusting sample loading volumes, making sample processing more efficient.

For QC of different sample types, the ProteoAnalyzer demonstrates strong compatibility and excellent reproducibility across a wide variety of complex biological sample types. The ProteoAnalyzer is especially beneficial to large-cohort sample studies, enabling rapid generation of consistent high-quality QC data and rapid, automated batch analysis. In addition, the protein quantification using the ProteoAnalyzer was successfully integrated into Majorbio's proteomics workflow, simplifying their process and enhancing data reliability. In summary, the ProteoAnalyzer empowers high-throughput proteomics laboratories by providing reliable support for efficient, high-quality, and cost-effective operations for protein sample QC.

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