

Application Forum

The New Face of Electrophoresis: Modernization of a Workhorse Technology

Mary Grace Brubacher

Bio-Rad Laboratories, Hercules, CA, USA

HISTORY OF ELECTROPHORESIS

Protein and nucleic acid electrophoresis is one of the most widely used tools in research. It is so commonplace in labs today that few people recall the origins of this ubiquitous tool. No other technique provides as much information about the purity of a sample and the sizes of its components as quickly and with as little effort and capital expense.

Protein electrophoresis was first developed in the 1930s, using sucrose and starch as separation media. In the late 1950s, improvements such as the development of polyacrylamide gels offered the ability to control pore size in a stable matrix and, as a result, provided better resolution. Electrophoretic analysis became widely accepted as a protein analytical tool after the development of denaturing, discontinuous stacking systems (1–4) in the mid- to late 1960s and the availability of commercial slab gel systems in the early 1970s. Eventually, the technology was adapted to nucleic acids, which advanced even further with the development of DNA sequencing methods and agarose gel systems in the late 1970s.

While the basic science and technology of electrophoretic analysis have changed little over the last 30 years, the development of low-cost, easy-to-use, reliable commercial systems has greatly improved the resolution, accuracy, and reproducibility of both protein and nucleic acid electrophoresis. Some good examples are mini format gel systems and precast gels, such as Bio-Rad's PROTEAN® and Criterion™ product lines, which

can resolve 5–250 kD proteins. Electrophoretic analysis of nucleic acids has also become reliable, easy, and reproducible due to the convenience and commercial availability of agarose gel systems and precast agarose gels, such as Bio-Rad's Sub-Cell® line.

FASTER RESULTS THROUGH MINIATURIZATION

The development of microfluidics technology over the last 15 years, such as the LabChip technology from Caliper Life Sciences, has enabled the automation of electrophoretic analysis of proteins and nucleic acids. The Experion™ system from Bio-Rad (Figure 1) implements and tailors this technology to provide fast, accurate, and high-resolution separations. It automatically performs the multiple steps of gel-based electrophoresis: separation, staining, destaining, band detection, imaging, and data analysis. Automated electrophoresis has its origins in capillary electrophoresis but does not require the same level of expertise as capillary electrophoresis and is performed more quickly. Very little hands-on time is required to set up and run an automated electrophoresis system, which enables the analysis of 10 to 12 protein, RNA, or DNA samples in as little as 30 minutes with great reproducibility. The Experion system removes the shortcomings of manual electrophoretic analysis by reducing the setup time, time to obtain results, and level of expertise required to obtain accurate, reproducible results. Additionally, because of the reduced volume needed to load and run a microfluidic chip, there is less waste of precious samples and reagents. While this automated system costs more than manual systems, it is a cost-efficient solution for laboratories that need to perform quick, repetitive electrophoretic analyses.

AUTOMATED ANALYSIS OF PROTEIN PURITY

One of the most appropriate applications of automated protein electrophoresis is to accelerate process development by monitoring column fractions during chromatographic purification (Figure 2). This can apply to checking the purity of a monoclonal antibody in an industrial quality control setting or following the purification of an engineered polyhistidine-tagged protein. The ability to have protein sizing, quantitation, and purity information all within 30 minutes allows for quick



Figure 1. The Bio-Rad Experion automated electrophoresis system.

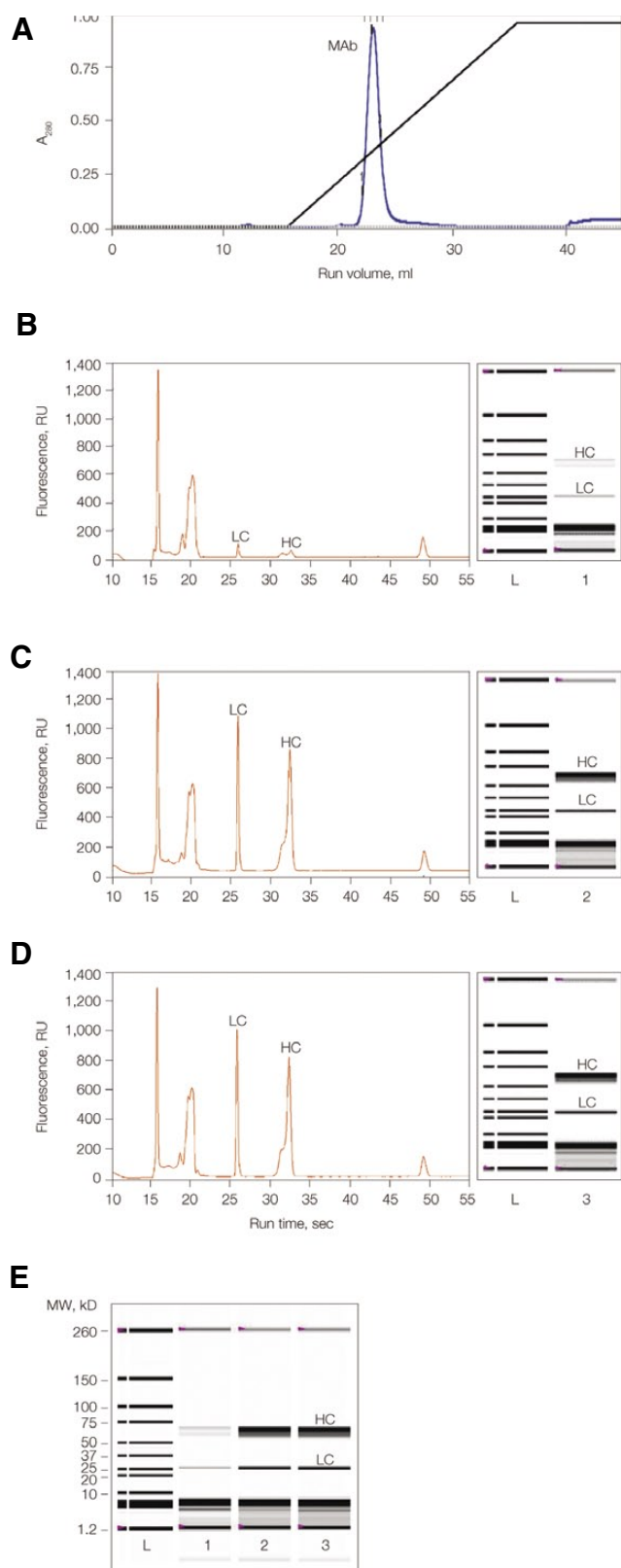


Figure 2. CHT™ ceramic hydroxyapatite purification of protein A-purified murine IgG (MAb). **A.** Chromatogram generated by BioLogic™ DuoFlow software showing the CHT-purified antibody. Three fractions, designated 1, 2, and 3, were chosen for Experion analysis. Blue trace, A_{280} ; black trace, NaCl gradient. **B–D.** Experion Pro260 analysis showing the electropherogram (left) and virtual gel images (right) of fractions 1 (B), 2 (C), and 3 (D). L, Pro260 protein ladder; right lane, MAb-containing fraction. **E.** Composite gel image comparing all three fractions. HC, heavy chain; LC, light chain.

identification of the most important fractions to pursue and enables faster fine-tuning of purification methods.

AUTOMATED ASSESSMENT OF RNA INTEGRITY

RNA integrity plays a major role in the generation of accurate quantitative results from gene expression analysis experiments (5, 6). cDNA made from RNA that has been degraded will not become amplified or labeled to the same extent as cDNA made from intact, undegraded RNA. For example, in RNAi or other studies using RT-qPCR, using degraded or even partially degraded mRNA as a starting sample can dramatically increase threshold cycle (C_T) values. These high C_T values will be misinterpreted as low levels of transcripts by as much as 1000-fold. Since different gene transcripts may be degraded at different rates, starting with a degraded mRNA sample could generate erroneous conclusions regarding the differential expression of various genes (6). Using degraded mRNA can also alter measured gene expression ratios 10-fold or more in microarray experiments (6).

Traditionally, RNA integrity is monitored by electrophoresis on a denaturing agarose gel. While this method is easy and inexpensive, it requires large amounts of sample, it involves the use of toxic chemicals, and interpretation of the data is neither straightforward nor accurate. Electrophoretic analysis of RNA is generally combined with a spectrophotometric quantitation and assessment of RNA purity, requiring more RNA sample to be used. The Experion automated electrophoresis system allows precise evaluation of the quality and the integrity of an RNA sample in a single step using as little as 25 ng of total RNA. Because the Experion system can directly quantitate and compare levels of 18S and 28S rRNA, the interpretation of the data is very accurate, unlike qualitative visual assessment of staining intensities of RNAs on a gel. Figure 3 provides an example of how the Experion system can be used to quickly provide an accurate estimation of RNA integrity, which can, in turn, ensure the accuracy and reliability of reported results (7).

A TIME AND A PLACE FOR MANUAL AND AUTOMATED ELECTROPHORESIS

Manual and automated systems both have benefits and drawbacks. Although manual systems require a significant amount of hands-on time, they allow the researcher to perform postseparation processes, such as blotting or band recovery. Once a sample has been applied to an automated electrophoresis microfluidic chip, it cannot be recovered following separation. Additionally, though microfluidic chips use smaller volumes of samples and reagents, this makes them less tolerant of buffer contaminants. Thus, manual methods are best suited for applications in which postseparation analysis or processing is required and in which flexibility, for example, in terms of the ability to vary the gel composition (gradient vs. fixed percent-

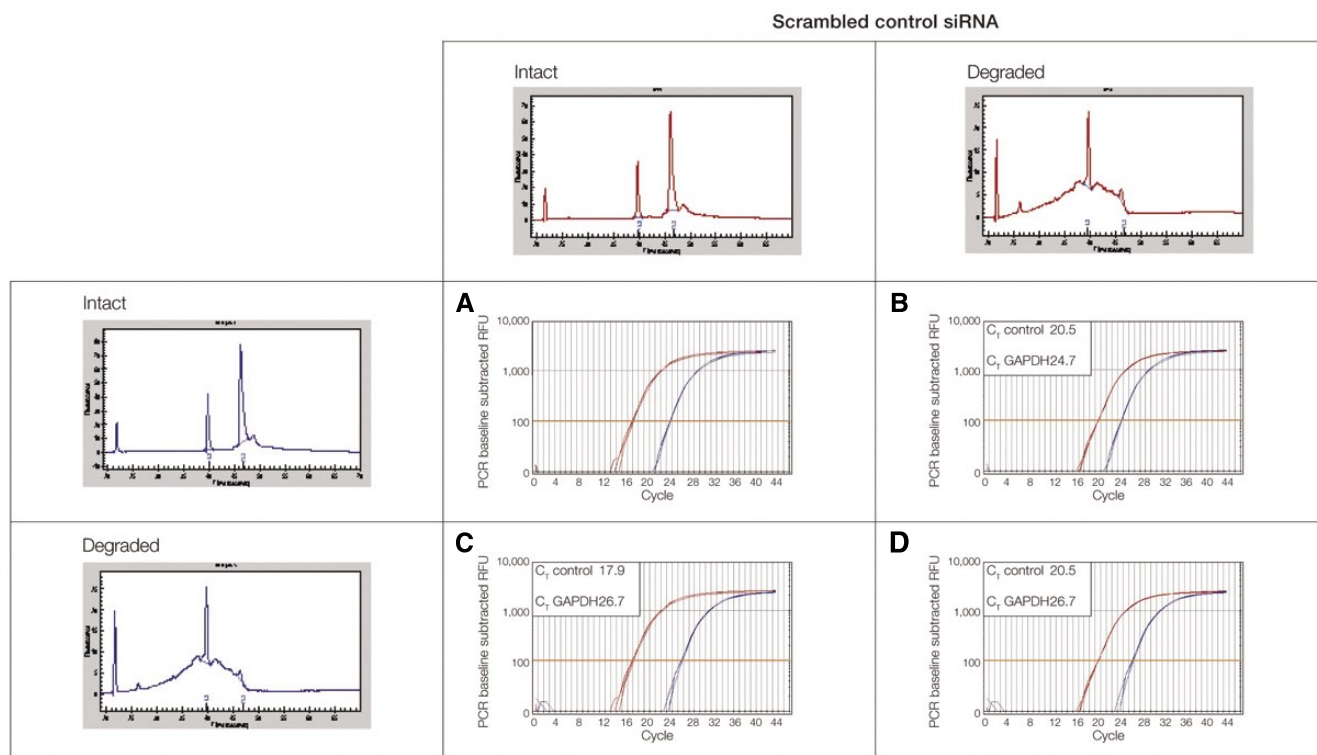


Figure 3. Analysis of RNA integrity and impact of sample quality on RT-qPCR. Experion electropherograms of 100 ng of intact or partially degraded total RNA from transfections of HeLa cells with control scrambled siRNA and GAPDH-targeted siRNA are shown. Panels A–D show RT-qPCR traces obtained using intact or degraded RNA from the control scrambled siRNA transformed cells (red) and from the GAPDH-targeted (blue) cells as a template as indicated in the electropherograms positioned above and beside the traces. Also shown are the average measured C_t values for each sample and the overall level of gene silencing, which is represented by the ΔC_t value.

age), is crucial to the lab's operation. Automated electrophoresis is best suited to a lab that performs repetitive analyses, has high labor costs or small amounts of precious samples, or needs the speed and convenience offered by an automated system with digital data storage for record keeping and reporting. Being able to perform both manual and automated electrophoresis is a realistic approach, because each method offers such distinct advantages.

CONCLUSIONS

Electrophoresis has evolved into one of the most ubiquitous analytical techniques in the life sciences. After more than 30 years of development and refinement, manual electrophoresis systems have attained a high level of reliability, resolution, and accuracy due to their ease of use and improved design. Automation is the latest stage in this process of evolution, and it provides unparalleled ease of use and reproducibility and reduced hands-on time and time to results.

The application of automated electrophoresis to both protein and RNA analysis has dramatically improved the ability to develop processes for protein purification and to ensure the accuracy of RT-qPCR, microarray, and siRNA-mediated gene silencing experiments. Selecting which option makes sense depends upon how electrophoresis will be used in your lab and that an upgrade path exists from manual to automated electrophoresis.

For more information about automated electrophoresis, visit us on the Web at www.bio-rad.com/ad/experion/

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