

Dorian M. Raymer
Douglas E. Smith

Department of Physics,
University of California,
La Jolla, CA, USA

Received May 26, 2006
Revised August 7, 2006
Accepted August 17, 2006

Research Article

A simple system for staining protein and nucleic acid electrophoresis gels

Researchers in molecular biology spend a significant amount of time tending to the staining and destaining of electrophoresis gels. Here we describe a simple system, costing ~\$100 and taking ~1 h to assemble, that automates standard nucleic acid and protein gel staining protocols. Staining is done in a tray or, with DNA gels, in the electrophoresis chamber itself following automatic detection of the voltage drop. Miniature pumps controlled by a microcontroller chip exchange the necessary solutions at programmed time intervals. We demonstrate efficient and highly reproducible ethidium bromide and methylene blue staining of DNA in agarose gels and Coomassie blue and silver staining of proteins in polyacrylamide gels.

Keywords:

Destaining / DNA / Electrophoresis / Protein / Staining DOI 10.1002/elps.200600325



1 Introduction

Gel electrophoresis is a powerful and ubiquitous method for analysis of DNA, RNA, and protein samples. One of the few downsides of this technique is that it is time consuming. Indeed, significant efforts have been made to developing more rapid processing methods such as CE and “lab on a chip” systems [1, 2]. While such methods are promising, the majority of researchers nevertheless continue to use classical techniques, which are often favored on the basis of their familiarity, reliability, and low cost. Given the wide use of these established techniques, simple improvements in their implementation may be of great value.

Staining of gels involves time-consuming manual processing steps. A researcher must repeatedly return to the bench to change solutions. The situation becomes burdensome when one wants to process multiple gels of different types in parallel with different starting times. Standard protocols are given in reference books such as “Molecular Cloning: A Laboratory Manual” [3] and “Short Protocols in Molecular Biology” [4]. Ethidium bromide staining of DNA in agarose gels typically involves removing the gel from the electrophoresis chamber, incubating it in the staining so-

lution for ~20 min and then in water for 30–60 min to destain, with several solution changes often being recommended to achieve the highest sensitivity. Effective staining of DNA gels with methylene blue, preferred by some for its low toxicity and capability for visualization without UV light, generally requires thorough destaining for good results.

Polyacrylamide protein gels are often visualized with Coomassie blue or silver staining. Silver staining, in particular, involves a large number of processing steps that require accurate timing. A typical protocol that works well in our experience calls for incubation in a fixing solution for 15 min, in water three times for 5 min, in a sensitizing solution for 15 min, again in water three times for 5 min, in silver nitrate for 30 min, in water and developer solution for 1 min, in developer for ~10 min, and finally addition of a stopping solution.

Here we describe a simple system for automating such protocols. We programmed a microcontroller chip to actuate miniature fluid pumps to add and remove solutions at specified time intervals. The system was built from inexpensive off-the-shelf components. While several commercial systems for automating certain gel staining protocols are available (*e.g.*, from Amersham and BioRad), these systems are ~50 times more expensive than the one described here and are less flexible. Furthermore, we describe a novel method whereby horizontal slab gels may be automatically stained in the electrophoresis chamber following automatic detection of the voltage drop at the end of electrophoresis.

Correspondence: Professor Douglas E. Smith, Department of Physics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

E-mail: des@physics.ucsd.edu

Fax: +1-858-534-3501

2 Materials and methods

2.1 Instrument design

Our system is shown schematically in Fig. 1. Model aircraft fueling pumps were used because of their low cost (\$15 each) and chemical resistance. The pumps were arranged to transfer solutions between reagent bottles and the staining chamber *via* silicone tubing. One pump was used as a circulator to periodically flow the solutions over the gel to insure efficient mixing. With larger gels, we found that the best results were obtained by using two circulators and arranging the tubing to spray the solutions across the width of the gel. Also we found it helpful to sandwich the gel between two screens to prevent it from folding during the staining and destaining process. Further details and a diagram are given in the Supporting Information. We note that this method eliminates the need for agitation by a shaker, which typically costs $\sim 10 \times$ as much as our entire system. We programmed an inexpensive microcontroller chip (PIC 16F628A, cost: \$2) to switch the pumps on and off at specified time intervals. The microcontroller was mounted on an inexpensive pre-assembled circuit board that provides relay switches and an interface for programming. Programs were written in Basic and compiled into machine code. A detailed parts list, assembly instructions, and program codes are given in the Supporting Information.

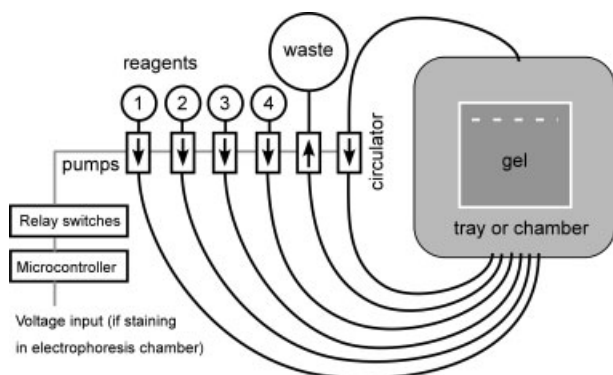


Figure 1. Schematic diagram of the system. Pumps actuated by a microcontroller add, circulate, and remove reagents from the staining chamber.

2.2 Reagents and electrophoresis equipment

Agarose, *Tris*-acetate-EDTA (TAE) buffer, *Tris*-glycine-SDS (TGS) buffer, ethidium bromide, methanol, acetic acid, Brilliant blue R-250, silver nitrate, formaldehyde, citric acid, and sodium thiosulfate were purchased from Fisher Scientific. Methylene blue and sodium carbonate were purchased from Sigma. λ DNA *Hind*III markers were purchased from Promega. PageRuler unstained protein markers were purchased from Fermentas.

Agarose slab gels (7×9 cm²) were run in a horizontal mini-gel system (Fisher Scientific #FB-SB-710). SDS-PAGE mini-gels (10×7 cm²) were purchased from BioRad and run in a vertical mini-gel system (BioRad Mini Protean III system). Larger 16×16 cm SDS-PAGE gels were purchased from BioRad and run in a vertical system (BioRad Protean II system). Protein mini-gel staining was done in the lid of a pipette tip box (13×11.5 cm², filled with ~ 150 mL of solution) (Fisher Scientific #02-707-325), the larger PAGE gels in a tupperware container (19×32 cm², filled with ~ 600 mL of solution), and DNA gel staining was done in the electrophoresis chamber (Fisher Scientific #FB-SB-710, filled ~ 300 mL of solution). Ethidium-stained gels were visualized on a UV transilluminator and methylene blue gels with a white-light converter plate and photos were taken with a Nikon Coolpix 995 digital camera. SDS-PAGE gels were imaged with a Canon Canoscan 3000ex scanner.

3 Results

3.1 Nucleic acid gels

To demonstrate that our system could stain agarose DNA mini-gels, we implemented ethidium bromide and methylene blue staining protocols, as described in Supporting Information. As shown in Fig. 2, our system gave results comparable to those obtained when staining gels manually. To demonstrate automatic staining in the electrophoresis chamber following the completion of electrophoresis, we wired the electrophoresis power supply to an opto-isolated digital input sensor on our circuit board. The microcontroller was then programmed so that staining and destaining began immediately after the end of the electrophoresis run was detected. The gels shown in Fig. 2 were processed in this manner. In this method, the gel sits in the horizontal electrophoresis chamber after running, and the staining and destaining solutions are circulated over the top surface of the gel to facilitate even staining. This approach allows the user to simply start an electrophoresis run and return later to retrieve the stained and destained gel. If desired, one may also use a separate staining tray.

3.2 Protein gels

We implemented standard Coomassie blue and silver staining protocols for SDS-PAGE mini-gels, as described in Section 2 and in Supporting Information. Photographs of processed gels are shown in Fig. 3 and indicate good results. As discussed above, silver staining involves many time-sensitive steps and particularly showcases the advantage of the automated system. Seven pumps were programmed to automatically deliver, circulate, and drain the fixing, rinsing, sensitizing, silver nitrate, developing, and stopping solutions.

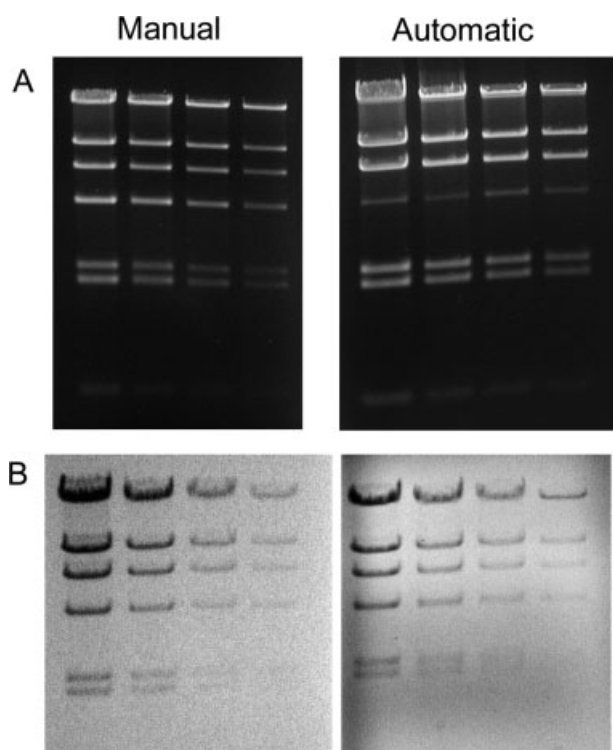


Figure 2. Comparison between agarose DNA mini-gels stained manually and automatically. (A) Ethidium bromide, with 250, 130, 60, and 30 ng of λ *Hind*III marker. (B) Methylene blue, with 1500, 750, 400, and 200 ng of λ *Hind*III marker.

4 Discussion

We have demonstrated a simple and inexpensive system for staining of nucleic acid and protein electrophoresis gels, thus automating a time-consuming task that is often done manually by researchers in molecular biology. The automated system yields results as good as those achieved manually and with increased reproducibility. Ready-to-use program codes for ethidium bromide, methylene blue, Coomassie blue, and silver staining are provided in the Supporting Information. The programs can be easily modified to implement other protocols. As this system is very inexpensive it is also practical to set up multiple stations for processing many gels with different protocols in parallel.

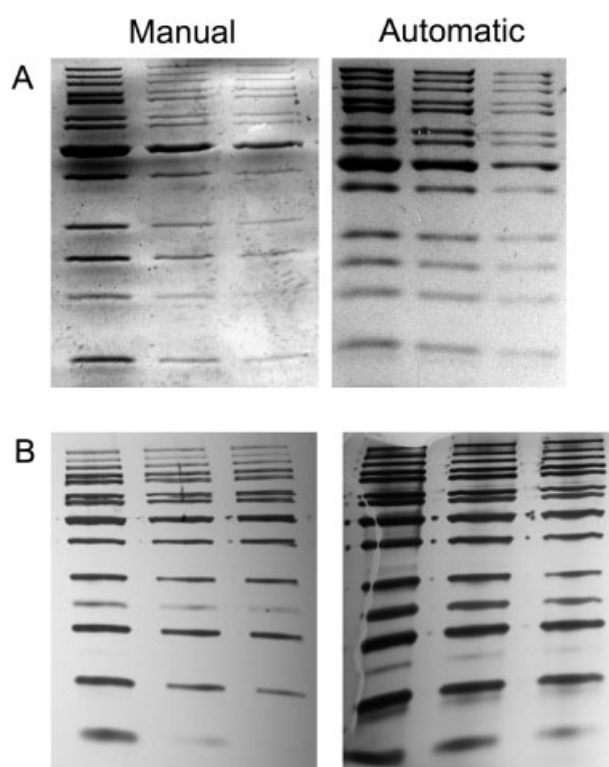


Figure 3. Comparison of SDS-PAGE protein mini-gels stained manually and automatically. Results with larger format SDS-PAGE gels were similar and are shown in the Supporting Information. (A) Coomassie blue, with 360, 180, 90, and 45 ng of protein marker. (B) Silver-stained with 60, 30, 15, and 7.5 ng of protein marker.

5 References

- [1] Hawtin, P., Hardern, I., Wittig, R., Mollenhauer, J. *et al.*, *Electrophoresis* 2005, 26, 3674–3681.
- [2] Landers, J. P., *Anal. Chem.* 2003, 75, 2919–2927.
- [3] Sambrook, J., Russell, D. W., *Molecular Cloning: A Laboratory Manual*, 3rd Edn., Cold Spring Harbor, New York 2001.
- [4] Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D. *et al.*, *Short Protocols in Molecular Biology*, 5th Edn., John Wiley, Indianapolis, IN 2002.