A Simple Homemade Apparatus for Real-Time Visualization of Nucleic Acid Electrophoresis That Saves Time, Optimizes Separation, and Reduces Chemical Risk

To the Editor:

Agarose slab gel electrophoresis continues to provide a low-cost alternative and a valuable complement to more sophisticated techniques for estimating the size of DNA molecules after restriction enzyme digestion, for evaluating PCR products, and for separating restricted genomic DNA or RNA before Southern or northern blot analysis (1, 2).

With traditional apparatus, the electrophoresis run must be interrupted before the agarose gel can be moved onto a UV transilluminator (wavelength approximately 300–360 nm), which is connected to a conventional or digital camera and placed in a dark environment for best results. This procedure is repeated until a satisfactory separation is achieved.

We have devised a simple, easily reproducible device (Fig. 1) that contains the following main components: (a) an electrophoresis chamber; (b) 2 UV light-emitting lamps (302 nm), which are placed below the plastic plate holding the gel; and (c) a commercial Web camera connected to a personal computer. The device can be opened to pour or remove a gel and to fill the sample wells. It is configured for connection to an external power supply and is equipped with a safety switch that shuts off the power when the device is opened. The apparatus was built with commercially available materials and may be easily reproduced at a moderate cost. Technical drawings, a list of materials used, and relative costs are available free of charge from the corresponding author.

The assembly of these components into a single unit makes our device different from the traditional apparatus; however, its key feature is the part of the electrophoresis chamber holding the gel tray, which is made of UV-transparent Plexiglas (4 mm; Rohm and Haas). This simple modification, combined with the positioning of the UV lamps under the electrophoresis chamber and the availability of a digital camera inside the apparatus, makes it possible to visualize and record separations of nucleic acids without interrupting the run and having to move the gel from the chamber.

Our apparatus has 3 substantial advantages compared with traditional devices. First, the visualization and recording of results is shortened and simplified. Digitalizing of the electrophoretic separation is accomplished by simply turning on the video camera and capturing the image. This procedure takes only a few seconds. Second, the separation of DNA molecules is easily optimized. In fact, DNA can be visualized at any moment of the electrophoresis run without interrupting it, allowing

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References


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selection of the best electrophoretic separation among those recorded. Third, the chemical risks to the operators are reduced. By making unnecessary any movement of the gel in and out of the chamber, use of our apparatus minimizes laboratory contamination and operator contact with the dangerous chemical agents added to the gel (e.g., ethidium bromide), loaded with the samples under analysis (e.g., SYBR® Green I) to make DNA visible under a UV source, or used to denature RNA (formaldehyde). Furthermore, the described apparatus may provide a basis for further configurational developments. In this regard, the possible implementation of recently developed DNA-detection systems based on UV light– or ultrabright green light–emitting diodes could provide further advantages in terms of sensitivity, throughput, and resolving power [e.g., (3)].

A commercial alternative to our apparatus is the FlashGel® system from Lonza Rockland (www.lonza.com). Compared with the FlashGel system, our apparatus has some substantial advantages; in particular, it is possible to carry out prolonged runs for increased separation of DNA fragments through of the use of larger gels [140 mm (length) × 150 mm (width) × 20 mm (height) vs 70 mm (length) × 84 mm (width) × 20 mm (height)] and allowing a free choice of agarose type and concentration for better adaptation of a given protocol to the range of molecular weights of the molecules to be separated; however, the electrophoresis time required for satisfactory separation with our device is at least 4-fold longer than for the
commercial device. This disadvantage is partially offset by the fact that our gels can accommodate 80 samples per run (compared with 34 for the FlashGel device). Furthermore, our method is markedly less expensive than the FlashGel device with respect to both the apparatus (613 vs 991 euros) and the agarose gels (0.08 vs 0.37 euros; cost per sample for electrophoresis on a 22-g/L NuSieve® agarose gel from Lonza Rockland).

Our apparatus is currently being used for various applications, including checking the size and quality of DNAs extracted from biologic materials (e.g., blood and saliva), PCR products of mitochondrial DNA before sequencing, and RFLP typing of polymorphisms within the IRS1 (insulin receptor substrate 1) and MCM6 (minichromosome maintenance complex component 6) genes (4, 5).

This newly designed apparatus can be easily reproduced and used in laboratories where analytical and preparative agarose slab gel electrophoresis of nucleic acids is carried out. It has substantial advantages in terms of saving time, optimizing separations, and reducing chemical risks to the operators.

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**Reference Values for Plasma B-Type Natriuretic Peptide in the First Days of Life**

**To the Editor:**

The clinical relevance of B-type natriuretic peptide (BNP) and the amino terminal fragment of its prohormone (NT-proBNP) as biomarkers in pediatric heart disease have recently been shown (1, 2). BNP and NT-proBNP concentrations are dependent on age and sex, at least in adults (3). Because commercial methods are affected differently by the presence in plasma of several peptides derived from the degradation of intact prohormone and BNP (3), little if any agreement exists among reported reference intervals, especially those used for infants during the first days of extrauterine life (1).

Data are scarce regarding the reference values for BNP and NT-proBNP in infancy (1, 2, 4). Recently, Nir et al. (2) summarized NT-proBNP concentrations measured with an electrochemiluminescence immunoassay method in 690 healthy individuals (47% males) with ages ranging from birth to 18 years, including 127 newborns in the first week of life (43 in the first 2 days). The NT-proBNP concentrations were highest in the first days of life, and then showed a marked decline. Concentrations in males and females differed only for children age 10–14 years.

Large reference value studies for BNP for newborns and infants have not been reported. To assess the reference values for BNP in the first days of life, we measured the peptide concentrations in residual EDTA plasma from blood samples

1 Nonstandard abbreviations: BNP, B-type natriuretic peptide; NT-proBNP, amino terminal fragment of the BNP prohormone.