BIOCHEMISTRY AND MOLECULAR BIOLOGY EDUCATION
Vol. 32, No. 5, pp. 341–347, 2004

Laboratory Exercises

Simple Protocol for Secondary School Hands-on Activity

ELECTROPHORESIS OF PRE-STAINED NUCLEIC ACIDS ON AGAR-AGAR BORATE GELS

Received for publication, January 20, 2004, and in revised form, June 8, 2004

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An extremely simple, inexpensive, and safe method is presented, which emulates nucleic acids isolation and electrophoretic analysis as performed in a research environment, in the context of a secondary school hands-on activity. The protocol is amenable to an interdisciplinary approach, taking into consideration the electrical and chemical parameters of the electrophoretic system. Furthermore, the laboratory is framed in a more comprehensive pedagogical setting, which addresses the methodological aspects of a pivotal scientific enterprise such as the Human Genome Project. In this setting, the hands-on activity is complemented with animations, paper models, and discussions. Additionally, our results indicate that the use of borate buffer and agar-agar gels suits many of the experiments included in college-level laboratory activities, which currently make use of more expensive agarose gels and TBE or TAE buffers.

Keywords: Electrophoresis, DNA and RNA visualization, agar-agar, cationic stains, high school, Human Genome Project.

Gel electrophoresis is a widely and routinely employed technique in the analysis and preparation of nucleic acids in the research laboratory. Its use has been extended to university and college biochemistry and molecular biology courses. More recently, as the general public awareness of biotechnological issues and its impact on society increases, the work with DNA has reached the secondary school classroom.

In the research environment, gel electrophoresis of nucleic acids requires the use of hazardous and expensive reagents such as acrylamide (neurotoxic) and ethidium bromide (toxic and carcinogenic), which are only available from laboratory suppliers. In addition, the specialized equipment employed such as ultraviolet transilluminators, electrophoresis chambers, and high-voltage power sources also pose health risks.

The nature of university and college laboratory facilities and the age group of their students allow for the implementation of such techniques, provided that adequate safety measures are observed. On the other hand, the handling of these dangerous substances and equipment in the secondary school classroom would suppose an unacceptable risk. Therefore, the work with DNA in these settings calls for the use of alternative protocols. Some providers of science teaching materials offer a variety of electrophoresis kits for high schools, featuring allegedly nonhazardous components (whose nature is not disclosed). However, because of the high prices and the added shipping and customs expenses for many countries, they are out of reach for most educational institutions worldwide, especially those of developing countries.

On the other hand, reports of simple nucleic acids extraction procedures and electrophoresis using homemade apparatuses are available both in print [1–4] and on the web [5, 6]. Nevertheless, these protocols still require laboratory reagents such as agarose for gel casting, Tris-hydroxymethylaminomethane (Tris)[1] as buffer, and bromphenol blue as loading dye. We propose a simpler protocol for nucleic acids extraction from plant tissues and its visualization by gel electrophoresis, replacing these reagents with cheaper and readily available substances. Furthermore, our nucleic acids in-gel visualization mechanism is an innovative application of stains, which were previously used in a different manner and requires less time than previously reported protocols.

Outline and Rationale of the Method

Nucleic acids are extracted from kiwi fruit and resuspended in glycerol aqueous solutions of cationic stains. Samples are electrophoresed in agar-agar gels in a homemade chamber at low voltage, using borate as buffer. This extremely simplified protocol requires no hazardous materials nor specialized and expensive reagents or equipment, constituting thus an ideal teaching tool to be included in high school curricula. Plasmid DNA, although not indispensable, is used here in order to characterize the method.

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[1] The abbreviations used are: Tris, Tris-hydroxymethylaminomethane; TBE, Tris-borate-EDTA; TAE, Tris-acetate-EDTA.

TABLE I
Set-up and materials checklist

Materials checklist

The listed quantities inside parentheses are adequate for a 30-student class, divided into 6 groups. Each group performs a single extraction and each student does the alcohol precipitation and precipitate recovery steps individually. The samples from each group are run in the same gel.

- kiwi fruits (6)
- 1.5% sodium chloride-10% kitchen detergent solution in tap water (750 ml)
- plastic bags (6)
- knifes (6)
- coffee filters or filter paper (6)
- glass or plastic containers or beakers (6)
- glass tubes (30)
- racks for glass tubes (6)
- alcohol (ethanol or isopropanol) (150 ml)
- evaporating dish or small shallow plastic or glass container (30; alternatively, the bottom cut out from plastic bottles can be used for this end).
- basic stain (Methylene blue, Crystal violet, Victoria Blue, Safranin O) (25 ml of a 0.075 g/liter aqueous solution in 20% glycerol)
- borate buffer, 3.8 g/liter (approximately 6 liter, depending on the size of the electrophoresis chambers)
- agar-agar (approximately 5 g)
- electrophoretic chambers (6)
- styrofoam combs (6)
- 12 V AC/DC adaptors (500 mA max) (6)
- insulin syringes without needle, dropper or Pasteur pipettes (glass or plastic) (30)
- scanner, camera, light-box, or overhead projector

Activity set-up

The laboratory protocol may be organized in one session, or in multiple linked sessions, according to the following schedule (the allotted time between parentheses includes explanations and discussion of results):

1. Extraction of nucleic acids and preparation of samples for electrophoresis (45–60 min). The samples are stored in the freezer (or refrigerator) until next session.
2. Gel casting and discussion of the electrophoretic system properties and functioning (measure of voltage, observation of reactions on the electrodes) (1 h). The gel may be stored in the refrigerator or at room temperature overlaid with buffer.
3. Electrophoretic run, documentation, and discussion of results (2 h). The time allowed for sample migration can be used for other activities or to finish the discussion of results from the previous sessions.

MATERIALS AND METHODS

Table I lists the required materials and describes a possible organization for the laboratory. Note that the symbol "√" has been included in the following methods, which make reference to Table II (technical tips and troubleshooting). Please refer to them prior to the set up of the protocol.

Nucleic Acids Extraction from Plant Tissues

The fruit of kiwi (*Actinidia spp.*) was used as source of nucleic acids because of the high yield obtained in comparison to other sources (banana, onion, strawberry, apple, pea, and liver were assayed).

Protocol—

1. Peel and slice one kiwi fruit (50–100 g) and place it inside a plastic bag. √
2. Add to the bag a solution made by dissolving 1 teaspoon of table salt (~1.5 g) in ½ cup of a 1:10 dilution of ordinary kitchen detergent in tap water (~125 ml).
3. Close the bag with a knot (allowing the air out first) and thoroughly crush the fruit pieces. √
4. Filter the mixture through a coffee filter or filter paper into glass tubes or small glass containers. √
5. Precipitate the nucleic acids by gently adding at least an equal volume of alcohol (ethanol or isopropanol) to approximately half a tube of the filtered solution. √
6. Let the tube stand for at least 5 min, in order to allow the nucleic acids precipitate to rise to the top of the alcoholic phase and spool it with a wood, glass, or plastic rod. √
7. Place the precipitate on an evaporating dish or small shallow glass container and gently pour off the remaining alcohol, with the aid of an absorbent pad or tissue. √

Pre-electrophoretic Staining of Nucleic Acids

Several stains were employed: Methylene Blue, Crystal Violet (also called Gentian Violet), Safranin O and Victoria Blue B. The selection was made on the basis of their structure and expected cationic behavior at pH 9 (the pH of the electrophoresis buffer used) and their availability in our laboratory.

Protocol—

1. Prepare the staining solutions in 20% glycerol in tap water, at the following concentrations: 0.075 g/liter for Methylene Blue, Crystal Violet, and Safranin O and 0.85 g/liter for Victoria Blue B) (see next section for the discussion on the determination of these concentrations). √
2. Resuspend the nucleic acids obtained as stated above from 5 ml of kiwi filtrate in ~0.5 ml of the staining solution, with the aid of the rod. Increase this volume if needed, to reach a complete resuspension of the precipitate. √
3. For the plasmid sample (see plasmid preparation next), mix one volume of plasmid solution at a concentration range of 1–10 μg/μl, with an equal volume of staining solution (as stated above). The amount to be loaded depends on the dimensions of the wells.

Plasmid DNA Preparation

Plasmid DNA derived from commercially obtained pGEM (from Promega, Madison, WI) was prepared by the alkaline lysis method [7] and used as a control to characterize the electrophoretic system and staining method.

Gel Electrophoresis

A home-made electrophoresis apparatus was used. Even if similar devices have already been described [1], our chamber was specifically designed to circumvent the limitations posed by the simplicity of the protocol (see details in the legend of Fig. 1 and in "Results" and "Discussion").

Protocol—

1. For the electrolyte and buffer solution, prepare sodium tetraborate decahydrate (borax) at 3.8 g/liter. Although the initial pH (pH 9) of this solution can be adjusted closer to

TABLE II
Technical tips and troubleshooting

Extraction of nucleic acids
- Make sure the plastic bag used in step 1 is resistant.
- A blender or mortar can be used in place of the plastic bag.
- Be careful not to puncture the filter. If fruit pieces are detected on the filtrate, repeat the procedure with a new filter.
- The disruption and filtration steps should be performed as fast as possible, to avoid the action of nucleases.
- In some countries or states, regulations do not permit the use of ethanol in schools. Isopropanol (rubbing alcohol) can be used instead for nucleic acids precipitation.
- Although the present protocol indicates the use of room temperature alcohol (approximately 20°C), most protocols advise the use of ice-cold alcohol in order to maximize yield. If you should obtain no precipitate in this step, try this modification.
- Spoolable DNA is usually obtained from kiwi. In any case, the nucleic acids precipitate can be recovered from the alcoholic phase by sliding it over the walls of the tube into the evaporating dish, with the aid of the rod. Inoculation loops also work very well for this purpose.
- The same kind of paper used to filter the solution can be used to eliminate the alcohol from the pellet placed in the evaporating dish.
- The precipitate should not be allowed to dry completely because this would make its resuspension more difficult. Generally, air-drying for 10 min is enough.

Pre-electrophoretic staining of nucleic acids
- Glycerol is used to increase the density of the nucleic acids preparation for its loading in the gel. A concentrated solution of sucrose can be used in its place.
- Distilled water may be used for reagents preparation if available, but is only necessary when the quality of the tap water is not adequate.
- It is convenient to prepare stock solutions of the different stains (at a 5 g/liter concentration, for instance). In order to prepare the working solutions of Methylene Blue, Crystal Violet, and Safranin O, add 1 ml of the corresponding 5 g/liter stock solution (which can be measured with an insulin syringe or calibrated dropper pipette) to 66 ml of 20% glycerol aqueous solution. For Victoria Blue B, add 17 ml of the 5 g/liter stock solution to 83 ml of 20% glycerol aqueous solution. Stock solutions should be stored in dark glass bottles.
- Staining may display variability depending on the age of the stain powders used and on the storage time of stock solutions.
- The pre-stained samples may be stored in the freezer until their electrophoretic analysis.

Gel preparation and casting
- Make sure to use an uncapped or loosely capped microwave-resistant bottle (or Erlenmeyer) to heat the agar-agar solution.
- Usually, 2 min in the microwave at medium power is enough to dissolve 0.8 g of agar-agar in 100 ml of borate buffer (note that this should be adjusted for each microwave). Monitor this step to avoid spillages. Make sure the agar-agar is dissolved by carefully swirling the bottle and checking for visible particles. If needed, increase heating time.
- Be careful not to burn yourself when handling the hot solution and do not place the bottle on cold surfaces.
- Allow the solution to cool off until the bottle can be held with the hands, before pouring it into the casting tray.
- If a microwave is unavailable, the solution can be heated in a boiling water bath.
- A dark-colored tape can be placed on the bottom of the casting tray just below the comb, in order to see the wells more easily during the loading of the samples.
- Most protocols for nucleic acids electrophoresis place the comb closer to the cathode. However, in the proposed protocol is important to place it in the middle of the casting tray (see “Discussion”).
- Make sure that the comb does not move when pouring the agar solution and during the solidification process.
- The comb should be removed very carefully as the styrofoam may adhere to the gel and tear the wells.
- The gel casting tray can be placed in the refrigerator after pouring the agar-agar in order to accelerate its hardening.
- Gel concentration can be reduced to 0.4% in order to use less agar-agar. In this case, extra care should be taken to remove the comb.
- Both the agar-agar and the borate buffer can be reused after the run.

Electrophoresis
- Medicine droppers bought at a drugstore or supermarket can also be used for sample loading.
- If the nucleic acids preparation is not adequately resuspended, the sample might get out of the well when loaded or fail to enter the gel, once the electrical current is connected. If this should happen, add more staining solution to the sample, mix thoroughly and repeat the loading step.
- It may be useful to determine the actual electrical field achieved across the gel in each particular electrophoretic system. To do so, measure the ΔV by placing each test lead of a voltmeter touching opposing ends of the submerged gel and divide the resulting value by the length of the gel. Reducing the distance between the chamber electrodes will result in a grater field. In addition, the volume of the electrolyte solution overlying the gel can be reduced to maximize the electrical current flowing through the gel (see Fig. 1 and “Discussion”).
- When shorter running times are required, a variable voltage power source can be used in place of the 12-V adaptor. In this case, due precautions should considered to minimize the electrical hazard. Also, due to the electrostatic nature of the interactions between the basic stains and the nucleic acids, high voltages might not be compatible with this protocol.
- If copper electrodes are used, the anode should be cleaned periodically during the run, as the $Cu(OH)_2$ precipitate may interfere with the electrical flux. Also, keep in mind that copper wire is usually sold with an insulating plastic covering (generally transparent), which should be removed by flaming the wire. Alternatively, stainless-steel wire can be used.
- If significant voltage or pH variations are detected in the course of the run, the buffer reservoirs of the constructed chamber may be too small.

Documentation of results
- When using Methylene Blue, Crystal Violet, or Safranin O as stains, results should be documented as soon as possible (e.g. within an hour of the end of the run), because of their rapid diffusion. On the other hand, gels of nucleic acids stained with Victoria Blue B can be stored in a refrigerator for several days with no significant loss of staining.

neutrality, this is not necessary and no pH variation has been detected during the electrophoresis.

2. Prepare a 0.8% solution (0.8 g in 100 ml) of agar-agar (sold at food stores) in borate buffer.
3. Melt the agar-agar by heating the solution in a microwave oven. $\surd$
4. Seal the ends of the casting tray with tape, place the comb (cut out from styrofoam) in the middle of the casting tray, and pour the agar-agar solution. $\surd$
5. When the gel has hardened, remove the comb and the sealing tape, and fill the electrophoresis chamber with borate buffer, making sure the gel is submerged. $\surd$

6. Load the nucleic acids samples, pre-stained as stated above, near the maximum capacity of the wells, avoiding to overflow them. Insulin syringes (1-ml capacity, without needle), dropper, or Pasteur pipettes are used in the place of automatic micropipettors. Loaded volumes may vary depending on the dimensions of the combs (for instance, a $5 \times 5 \times 5$-mm comb tooth allows for a 50- to 100-μl load). In the neighboring wells, controls consisting only in the stains (without the nucleic acids) should be loaded for comparison. $\surd$
7. Connect the chamber electrodes to a 12-volt AC/DC adaptor (500-mA max), employed as power source (see next section for discussion about the electrical hazard and references to biosafety issues). An adequate electrical field value of 1 V/cm was achieved with our system, resulting in a perceptible nucleic acids migration in 1 h. $\surd$
8. When the desired migration of the samples is achieved, disconnect the power source, carefully dismount the gel, and place it inside a transparent plastic bag. In order to discuss the results, place the gel over a light box or overhead projector. A scanner, photocopier, or camera can be used to document the results. $\surd$

Agarose or agar-agar gels including ethidium bromide at a concentration of 0.5 μg/ml were run in parallel as controls and as a means of characterizing the proposed protocol in terms of sensitivity and resolving time spans.

RESULTS AND DISCUSSION

Nucleic Acids Extraction

Different sources can be used for the extraction of nucleic acids. Nonetheless, the idiosyncrasies of the different tissues may require variations in the basic protocol in order to solve problems related to disruption of cell walls, presence of nuclease activity, turbidity of the filtrate, etc. Moreover, possible health threats associated with the manipulation of animal tissues (prions and pathogenic agents) should be taken into account.

Using kiwi fruit as nucleic acids source, we found that the tissue is easily disrupted and results in a clear filtrate and a high yield of spoolable DNA. This may be due in part to *Actinidia spp*. polyploidy and the high concentration of proteases in its fruit, which may destroy nucleases. If using alternative sources, the nucleic acids extraction yield can be improved by heating the salt detergent solution to 60 °C for 10 min (in order to inactivate nucleases) or by adding meat tenderizer or contact lens cleaning solutions (which contain proteases) to the preparation. In our experience, strawberry constituted a suitable alternative source of spoolable nucleic acids, albeit the yield was not as high. The usual washing step with 70% ethanol before the resuspension of the precipitated nucleic acids proved not to be required when following this protocol (results not shown). While most protocols advise the use of ice-cold alcohol in order to maximize the nucleic acids precipitation yield, based on Ref. 8 and empirical results, we use room temperature alcohol ($\sim$20 °C).

Electrophoresis

The layout of the home-made electrophoretic chamber was designed to maximize the electrical current passage through the gel, contrary to traditional more simple chambers, in which the gel is submerged in a single tank and there are spaces between the gel and the walls of the chamber (see Fig. 1, *a* and *b*). Thus, even if the output voltage of the power source is maintained very low in order to avoid electric hazards, an adequate electric field of 1 V/cm is achieved and the electrophoretic separation of nucleic acids can be performed in reasonable time spans. If running times are to be further improved, the length of the gel (and consequently the distance between the electrodes in our design) can be reduced. Due to the electrostatical nature of the interactions between the basic stains and the nucleic acids, high voltages may affect the binding and their consequent detection.

Different metals may be used to manufacture the electrodes of the chamber. Although the use of electrodes made from metals with a high reduction potential such as platinum or stainless steel is generally advisable (as they are not altered and consumed when the current is connected), we prefer to use copper electrodes. In addition to being cheaper, the copper in the anode is oxidized to Cu^{2+} and a $Cu(OH)_2$ blue precipitate is formed at the pH of the borate buffer. On the other hand, the bubbles released at the cathode correspond to the H^+ reduced to gaseous H_2. These phenomena can not only be used to have the students identify the positive and negative poles in the system (as the electrodes are connected indistinctly to either end of the power source), but also to introduce or reinforce chemical concepts such as redox reactions, as part of an interdisciplinary approach.

In the same spirit, the electrophoretic system can be modeled in terms of an electric circuit (see Fig. 1, *b* and *d*, modified from Ref. 9). Note that the zone of the gel is represented by two parallel resistors, one corresponding to the gel itself and the other to the buffer layer above the gel. At constant voltage, the current across each component can be modified by altering the corresponding resistance. Thus, minimizing the height of the buffer layer above the gel (and thus maximizing the corresponding resistance), the electrical current through the gel is maximized. Likewise, by placing the electrodes near the ends of the gel, the effective voltage across it (ΔV) is improved (see Ref. 9 for a detailed treatment of the electrical parameters of an electrophoretical system).

Tris-borate-EDTA (TBE) and Tris-acetate-EDTA (TAE) are generally used as electrolyte/buffer solutions for agarose electrophoresis. Our protocol dispenses with Tris and EDTA. The borate buffer used (3.8 g/liter borax) is cheap, innocuous, provides an adequate ionic strength, and maintains the pH at 9 throughout the run (at the top end of the range in which nucleic acids are stable).

The use of agar-agar in the place of agarose further reduces the cost of the activity. However, if one would want to stain a gel with Methylene Blue or another cationic dye after the run, agar-agar would not be advisable because de-staining is extremely difficult, unless a very low concentration of the dye is used (however, this would call for a prolonged staining time and result in nucleic acids diffusion from the gel).

With the implementation of the staining method proposed here, this and other innocuous cationic dyes can be employed for nucleic acids visualization in agar-agar gels. Although the resolution of nucleic acids is sacrificed to a degree, the inclusion of a suitable control of the stain by

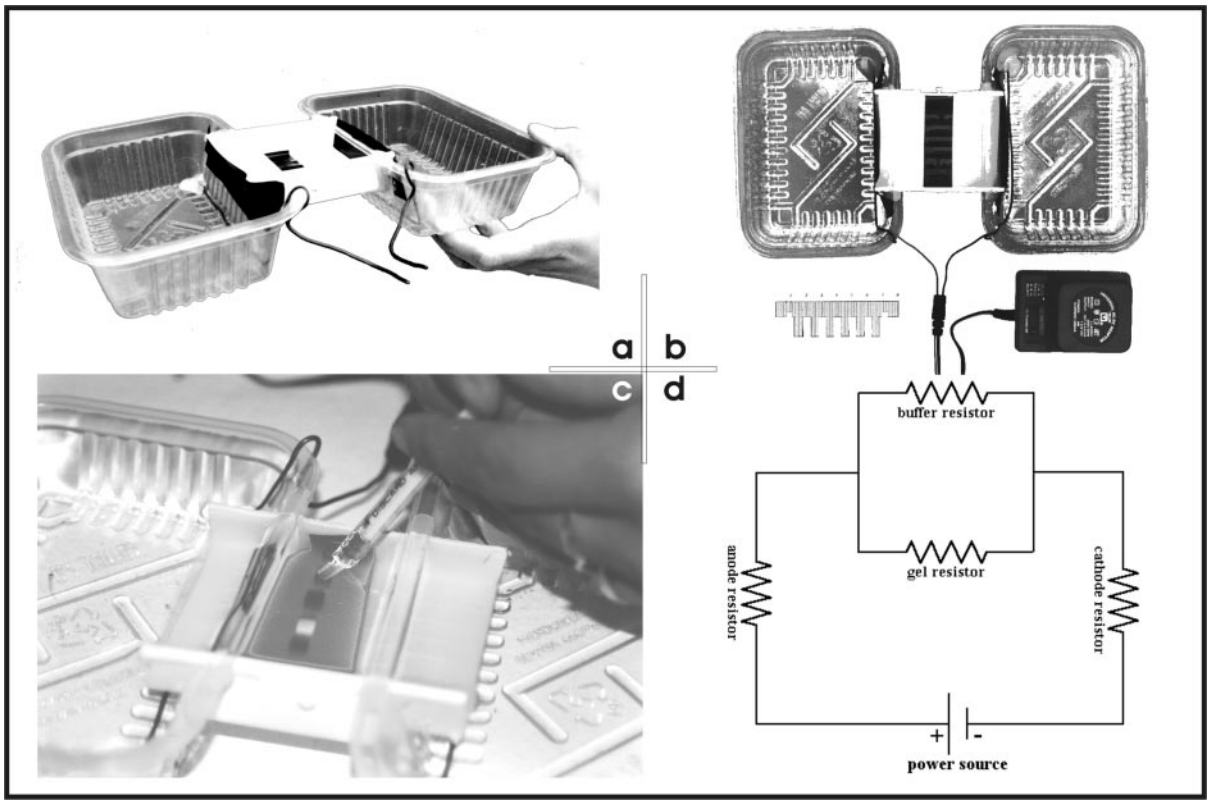

FIG. 1. **Home-made apparatus for horizontal gel electrophoresis.** Side view (*a*) and top view (*b*) of the device, which is connected to a 12 V/500 mA adaptor used as power source. The buffer reservoirs are made from rectangular plastic food containers, and the gel casting tray and platform that connects them is cut out from a plastic soap dish. The parts were assembled and glued with hot silicone. Note that the electrodes are placed on the side of the reservoirs immediately adjacent to the gel platform. The comb is cut out from styrofoam. As shown in *c*, samples are loaded using 1-ml insulin syringes without the needle. A scheme of an electrical circuit (*d*) representing each element of the system (*b*) is shown (modified from Ref. 9).

itself (this is why the comb is placed in the middle of the gel) allows to evidence the presence of nucleic acids in the sample and recognize a differential migration of plasmid, genomic DNA and RNA.

As shown in Fig. 2, the stain preparations generally move toward the cathode, with the exception of Victoria Blue B, which under the described conditions remained in the well. For the samples containing nucleic acids (from kiwi or plasmid), a smear is obtained in all cases, with differences in contrast, mobility, and definition of bands for each stain.

Fig. 3 compares the proposed method with the ethidium bromide standard method. Under the described conditions, Methylene Blue allows to clearly evidence the presence of nucleic acids contained in the preparation, although with a low sensibility threshold. While the detection limit of ethidium bromide reached 0.5 μg under our conditions (Fig. 3*b*, *lane 6*), that of Methylene Blue reached 6 μg (Fig. 3*a*, *lane 4*). The results are similar for the other cationic stains tested (results not shown). What appears to be a crucial point is the concentration of the positively charged stain relative to that of the nucleic acids in the sample. If this ratio is too high, this might hamper the electrophoretic motility of the macroion and even prevent it from entering the gel, as the force derived from the electrical field is not enough to counteract the frictional force imposed by the gel sieve. In this case, part of the stained material can be seen concentrated in the anode-side of the well. In Fig. 3 (*lanes 5* and *6*), the ratio of dye:nucleic acids concentration is too high, and this accounts for the observed pattern. For this reason, the concentration of the stain to be added to the sample should be empirically optimized taking into account the nucleic acids yield of different sources and protocols.

The pre-staining of the samples has a further advantage, making unnecessary the usually employed anionic loading dyes (such as xylene cyanol and bromphenol blue). On the other hand, plasmid DNA has been used here in order to characterize the method, but it is by no means indispensable to carry out the activity, as long as the concentration of the dye is adjusted for the nucleic acids samples obtained from the tissues.

Safety Issues

Safety constitute a most important issue to address when working at secondary school level, especially when chemicals capable of interacting with DNA are involved. If no information on their mode of action or potential health and environmental effects is available, these chemicals should be handled carefully and considered potential mutagens and carcinogens. The stains used in this report are basic in nature and therefore interact with DNA through electrostatical forces, as opposed to the intercalating nature of stains such as ethidium bromide, which is a known mutagen. Some of the basic stains included here have

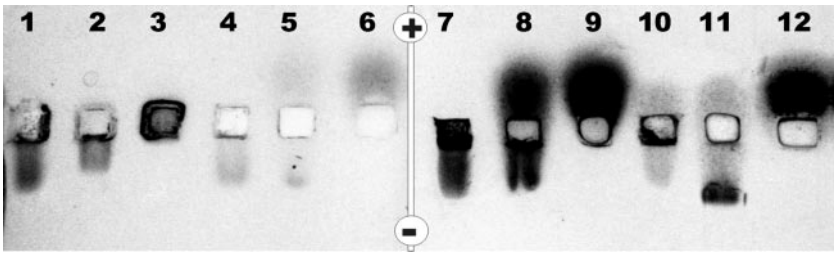

FIG. 2. **Electrophoresis of pre-stained nucleic acids on agar-agar borate gel.** Samples loaded as follows: nucleic acids extracted from kiwi (*lanes 1*, *4*, *7*, and *10*), plasmid DNA (*lanes 2*, *5*, *8*, and *11*), and stain alone (*lanes 3*, *6*, *9*, and *12*). The stains shown are: Victoria Blue (*lanes 1–3*), Safranin O (*lanes 4–6*), Crystal Violet (*lanes 7–9*), and Methylene Blue (*lanes 10–12*). The relative position of the electrodes is shown. The gels were photographed using a reflex photo camera with macro lens (and contrasted digitally using Adobe Photoshop 7.0). The gels were run for 1 h on home-made chambers at 12 V.

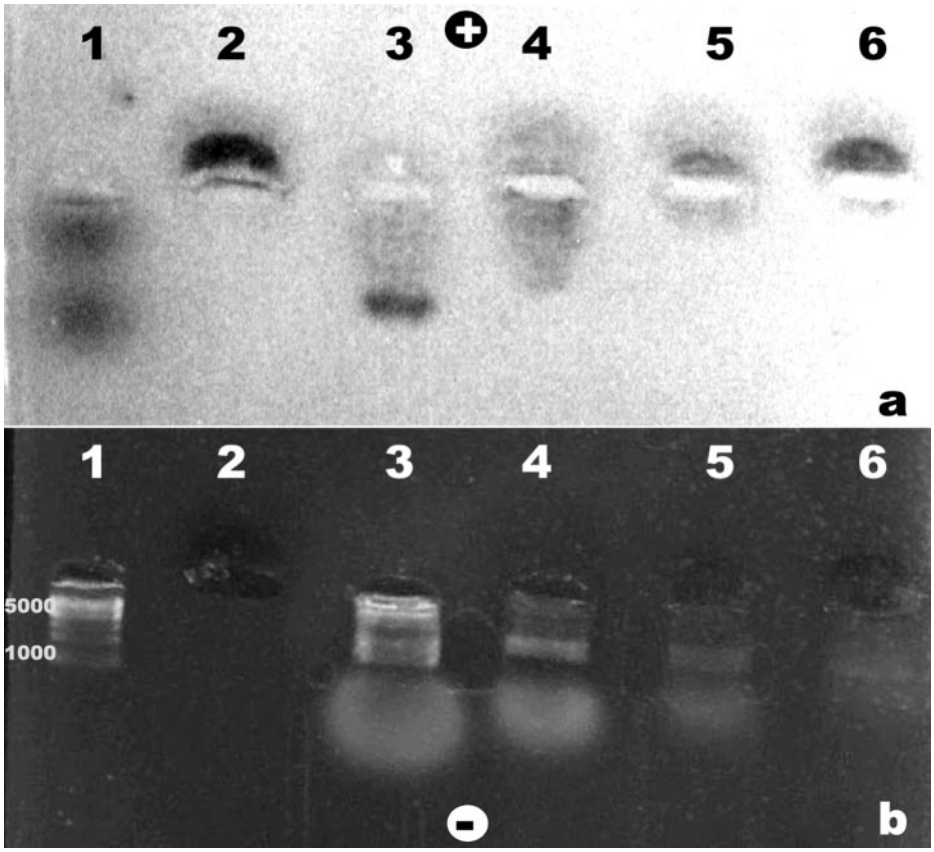

FIG. 3. **Analysis of the protocol of detection of nucleic acids.** Serial dilutions of a pGEM plasmid mini-preparation (not treated with RNase), pre-stained as described in the text with 0.075 g/liter Methylene Blue, were loaded on a 0.8% agar-agar borate gel and electrophoresed at 12 V for 1 h. After being photographed with a reflex photo camera (*a*), the gel was submerged in a 0.5-μg/ml ethidium bromide solution for 20 min with gentle shaking and photographed under ultraviolet light (*b*). The actual quantity of nucleic acids loaded (as determined spectrophotometrically for each sample and according to the supplier for the ladder) is as follows: *lane 1*, 1-kb ladder (New England Biolabs, Beverly, MA), 2.5 μg; *lane 2*, Methylene Blue, no nucleic acids; *lane 3*, 60 μg; *lane 4*, 6 μg; *lane 5*, 1 μg; *lane 6*, 0.5 μg. The colored bands in *lane 1* (*a*) correspond to bromphenol blue and Xylene Cyanol Blue of the loading buffer of the ladder. The photograph in *a* was contrasted digitally using Adobe Photoshop 7.0.

been extensively used for therapeutic ends. However, there are conflicting reports in some cases [10, 11] and not enough information about long-term effects in others (such as Victoria Blue B). Even if better characterized stains such as Methylene Blue should be used preferably, safety information about all reagents should be periodically updated. See Refs. 12 and 13 for reviews on staining mechanisms and classification of dyes. Following basic laboratory safety guidelines, students should never handle the stains in their powdered form, but diluted aqueous solutions of them in limited quantities instead. See Ref. 14 for an adequate treatment of safety issues regarding work with DNA.

Pedagogical Considerations

The proposed protocol can be further exploited if used as part of a more comprehensive pedagogical scenario. It has been used by us in a workshop addressed to pre-service and in-service teachers, which sought to emulate the process of obtaining the sequence of the Human Genome. Briefly, the research problem is divided into four distinct stages (which can be scheduled in a single or four or more linked sessions), as detailed in Fig. 4. The experimental work described here corresponds to the first stage. The second stage is approached using paper models of a simplified genome, which are cut with scissors representing different restriction nucleases (activity modified from Ref. 15). The subsequent cloning of digestion products into plasmid vectors is explained with the assistance of Shockwave Flash animations, obtained from the Library of Crop Technology Lessons developed at the University of Nebraska [16]. The third stage is also analyzed with the aid of computer animations from the Dolan DNA Learning Center [17], and an automated sequencing facility is visited. Finally, the above-mentioned paper model is used once again at the fourth stage, where di-

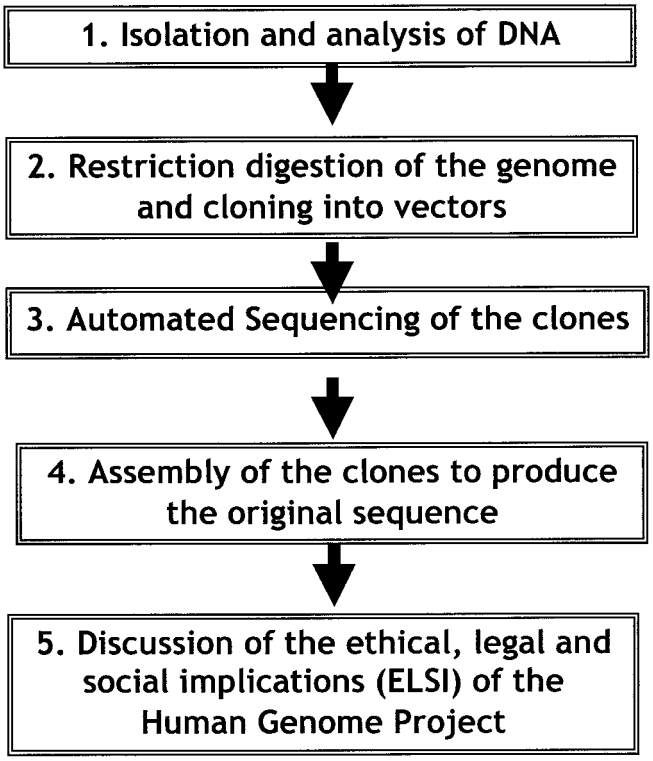

FIG. 4. **Proposed pedagogical setting: the obtention of the sequence of the human Genome.** The experimental protocol described in the article is used to mimic the first stage of this scientific enterprise. See text for further details.

gestion segments produced by two different enzymes are collated to assemble the original sequence. At this point, the use of bioinformatics tools for the analysis of sequence data is introduced in a lecture. A final session is devoted to the discussion of the ethical, legal, and social implications of the knowledge of the Human Genome, discussing diverse hypothetical and real situations.

For a general background on the theoretical and practical aspects of the topics covered, instructors and students can refer to textbooks and laboratory manuals [7, 8, 18–24].

CONCLUSIONS

The proposed method allows students to conduct a hands-on activity focusing on the extraction and analysis of nucleic acids from different tissues, which is amenable to any secondary school classroom as a first approach to the work carried out at a molecular biology laboratory. The protocol adheres to a high safety standard at a very low cost. All of the materials used can be obtained in fact from nonspecialized suppliers. Furthermore, distinct aspects of the electrophoretic technique are intended to be addressed from an interdisciplinary stand-point, which would foster the collaborative work of teachers lecturing different courses of the secondary school science syllabi. Overall, as part of a series of lectures, combining paper modeling, animations, and traditional explanations of related recombinant DNA techniques, the protocol serves the purpose of introducing secondary school students to a research scenario in the molecular biology and genetics fields.

Additionally, our results indicate that the use of borate buffer and agar-agar gels suits many of the experiments included in college-level laboratory activities, which currently make use of more expensive agarose gels and TBE or TAE buffers. Further analysis will be performed in order to determine whether these inexpensive materials are adaptable to other applications such as recovery of nucleic acids from the gels for further manipulation or transfer to membranes for hybridization.

Acknowledgments—We would like to thank the support provided by the Inspección Regional de Enseñanza Media - Ciclo Básico de Canelones and Insp. Elizabeth Mazzuchi in particular, and Inspección Nacional de Biología. We also acknowledge the constant assistance of their colleagues from the Biology Laboratory of the Centro Regional de Profesores -Prof. Vivian Cuns and Prof. Susana Vieira. Finally, we thank the secondary school teachers and pre-service teachers, which took part in the workshops during 2002 and 2003.

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