

GUIDELINES FOR SEPARATING DNA (Deoxyribonucleic Acid) USING GEL ELECTROPHORESIS IN HIGH SCHOOL LABORATORIES

General Introduction

Biotechnology can simply be defined as the manipulation of biological organisms to create useful products (Microsoft® Encarta® Online Encyclopedia 2003). For centuries, humans have routinely employed biotechnological methods in diverse fields such as medicine, agriculture and waste disposal. Some of the most current and fundamental advances in biotechnology have included genetic engineering (e.g., the creation of transgenic organisms), cloning and the production of monoclonal antibodies.

These applications of modern biotechnology had their inception from the landmark studies of Watson and Crick in 1953 on the biochemical structure of the deoxyribonucleic acid (DNA) double helix. Arber's discovery of restriction enzymes (special enzymes that can segment DNA at specific points) in 1960, and the application studies of using these enzymes by Cohen and Boyer in 1973 to remove segments of DNA from one bacterium and reinsert it into another, led to the development of recombinant DNA technology (or genetic engineering). An example of recombinant DNA technology involves the production of human insulin by incorporating the gene sequence for insulin production into bacterial plasmids. Genes (specific sections of the DNA) from different organisms (humans and the bacteria *E. coli*) are often combined *in vitro* to make a type of DNA called recombinant DNA. This recombinant DNA is often reintroduced into the prokaryotic cells where it can replicate and be expressed.

Other techniques used in DNA biotechnology include the creation of biodegradable plastics from lactic acid amalgamation produced from bacterial fermentation of corn stalks and the commercial production of factor VIII (a blood protein clotting factor) to help treat hemophilia. DNA technology has also helped scientists to study various molecular eukaryotic gene structures and functions that can be used to decipher the relatedness of various species in evolutionary history.

RATIONALE

The movement and separation of charged molecules in an ionic solution in response to an electric field is termed electrophoresis. Gel electrophoresis is a method used in molecular biology to separate macromolecules such as proteins and nucleic acids, (of which DNA is an example) based on physical properties such as their size, shape and electric charge. In molecular biology, gel electrophoresis is one of the standard, analytical, biochemical tools used to study genetic material such as recombinant DNA. As an analytical tool, electrophoresis is simple, rapid and highly sensitive. It can be used to study the properties of single charged molecular species; for example, using the technique of gel electrophoresis, it is possible to determine the evolutionary relationship among species of plants and animals, since it is possible to separate and identify protein molecules that differ by as little as a single amino acid.

Molecular genetics is a fundamental component studied in the field of molecular biology. High school students in Ontario who have registered for the Grade 12 Biology University Preparation Course (**SBI 4U**) will need various skills to understand various

biochemical and molecular tools that are routinely employed in molecular laboratories within a University setting. The molecular genetics strand in the SBI 4U course has been designed to be taught within a practical framework, in which gel electrophoresis is a recommended technique to study the components of the DNA molecule. There are also direct applications in exploring various specific expectations in the Grade 11 Science University/College Curriculum, **SNC 3M** (Strand: Technologies in EVERYDAY Life) and in the Grade 12 Science University/College Curriculum, **SNC 4M** (Strand: Science and Cont. Societal Issues).

The separation of DNA using gel electrophoresis involves a conceptual knowledge of the apparatus, the specific techniques involved (e.g. the use of restriction enzymes) and the safety measures that accompany the use of these materials and techniques. The intent of this article is to provide secondary educators with an overview of how to perform agarose gel electrophoresis techniques safely. As an analytical tool, gel electrophoresis is also rapid and sensitive hence accurate results can be generated within a short time period. Without background knowledge of the types of materials involved, the use of this rather simple tool can seem complex and daunting. This article also encompasses useful terminology and methodologies that are related to the technique of gel electrophoresis.

WHAT IS GEL ELECTROPHORESIS

Gel electrophoresis is one of the most widely used techniques that is used to separate macromolecules such as polypeptides and nucleic acids (DNA fragments or RNA,

Ribonucleic Acid). This biochemical method is successful in separating DNA since this macromolecule has charged groups that enable them to migrate in an electrical field (Solomon *et. al.*, 1999). All nucleic acids remain negative at any pH used in gel electrophoresis. In addition, the PO_4^- group of each nucleotide of the nucleic acid confers a fixed negative charge per unit length of molecule. The electrophoretic separation of nucleic acids is therefore distinctively according to size. Electrophoresis involves five components, the driving force which is the electric current, the sample to be separated (e.g., DNA), the support matrix (e.g., Agarose gels), the buffer (e.g., Tris EDTA¹ [ethylene diamine tetraacetic acid] buffer) and the detecting staining system (e.g., methylene blue and ethidium bromide²).

Since nucleic acids are negatively charged, both DNA and RNA will migrate through the gel in the direction toward the positive pole of the electric field. Since the gel acts as a sieve, it normally impedes the movement of larger molecules. Therefore smaller molecules will migrate faster along the gel toward the positive electrode (anode). The rates at which these molecules travel are inversely proportional to their molecular weight. The electrophoretic mobility of DNA through agarose gel is dependent on the molecular size of DNA. For example Linear DNA travels through the agarose gel matrix at rates inversely proportional to \log_{10} of its molecular weight. In order to determine accurately the molecular weights of the unknown fragments, all samples that are being

¹ EDTA is a chelating agent that binds and inactivates divalent ions such as magnesium. This is of fundamental important since nucleases require divalent ions to function. Nuclease can degrade DNA, hence their inactivation is important in gel electrophoresis.

² Ethidium Bromide (Et Br) is a known mutagen and a suspected carcinogen and is not used in high school classroom electrophoresis activities. However it is often used in research labs to stain separated DNA. A section on safety issues

analysed using gel electrophoresis will usually be run in parallel with known standards or DNA ladders (i.e. DNA fragments of known molecular weights).

USEFUL TERMINOLOGY:

1. ELECTROPHORESIS BUFFER: There are several buffers that are often recommended for electrophoresis of DNA. The commonly used buffers in electrophoresis are TAE buffers and TBE buffers (Tris-acetate-EDTA and Tris-borate-EDTA, respectively). Buffers are important they not only ensure an optimal pH to carry out gel electrophoresis, but they also provide ions to support conductivity.

2. Ethidium bromide (Et Br): Ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide) has traditionally been used for staining DNA and RNA in gels (Sinclair 2000). While the procedures for using EtBr are simple, EtBr is considered to be toxic. Ethidium Bromide is a flat aromatic chemical that intercalates between base pairs in the double helix of the DNA molecule. When ethidium bromide is bound to the bases of DNA, the complex will produce a fluorescent orange colour when irradiated with a transilluminator box (UV light). **WARNING:** Et Br is a suspected carcinogen and heritable mutagen. For full details one should consult the Material Safety Data Sheet (MSDS) for details.

surrounding the use of Et Br will be discussed to provide information on this aromatic molecule and alternative stains such as methylene blue that can be substituted during agarose gel electrophoresis activities.

Ethidium bromide is often used in agarose gel electrophoresis in research labs to stain separated DNA fragments. However, this compound can alter the biochemical structure of DNA by causing the mass of the fragments to change or the rigidity of the fragments to be altered. Some agarose gel electrophoresis protocols suggest that the ethidium bromide can be added to agarose gel before loading the DNA test mixture (that is to be separated) into the wells in the gels. Other protocols often stipulate that ethidium bromide be added after the DNA fragments have been electrophoresed, to circumvent the changes that can impact the mobility of the DNA fragments as they migrate along the agarose gel. The former method has an advantage of not requiring a soaking time following electrophoresis and also that the gel can be monitored using a hand-held UV light source.

- 3. DNA LADDER:** A DNA ladder is a mixture of DNA fragments (usually 10-20) of known size. The size of the DNA strands that are separated can often be determined by comparing their relative position to that of the DNA strands of the DNA ladder. Several DNA ladder mixes are commercially available.

- 4. Loading Buffer:** a buffer that contains 25% v/v glycerol and/or sucrose and a tracking dye. The tracking dye can be bromophenol blue that is used to render visible progress of the sample during electrophoresis.

- 5. Macromolecule:** A very large organic molecule, such as a protein or nucleic acid (DNA- Deoxyribonucleic Acid, RNA- Ribonucleic acid).

6. **Methylene Blue:** Methylene Blue and its oxidation products, such as Azures A, B and C, Toluidine blue O, Thionin and Brilliant cresyl blue are described as thiazin dyes. They are considered to be the safest dyes to use to stain DNA in high school classrooms. The exact mode of action of methylene blue as a stain is not known. It does not intercalate with DNA, but is thought to bind ionically to the negatively charged PO_4^- backbone of Nucleic acids. Thiazin dyes can therefore be used to stain DNA and single stranded RNA. When utilizing methylene blue, a solution diluted to 0.02-0.04% in water is suggested. The sensitivity of methylene blue is considerably less than Et BR. However if 50ng per lane is used with 1 hour staining and overnight destaining in distilled water and the results viewed with a UV **trans-illuminator** box, desirable results are achievable.
7. **Restriction Digestion and Restriction Enzymes:** The process of restriction digestion involves cutting DNA molecules into smaller pieces with the aid of special enzymes called restriction endonucleases (often referred to as restriction enzymes or RE's). These special enzymes recognize specific base sequences in the DNA molecule. Using the base sequence CTATTAG as an example, RE's will cut the DNA into smaller fragments at these specific sequences wherever they occur in the DNA molecule. Restriction enzymes usually digest DNA at their specific palindromic sequences.
8. **Spooling DNA:** DNA must be purified before it can be subjected to gel electrophoresis. All nucleic acids can be purified and concentrated using protocols

that involve precipitation with alcohol (either ethanol or isopropanol). Alcohol precipitation also removes salts in the buffer solutions, sugars and amino acids. Chromosomal DNA from bacteria can consist of 3 million base pairs and plasmids, which can be a few thousand. Since these are considered to be large molecules, it is possible to isolate the DNA using a technique called spooling. A simple protocol for spooling is outlined below.

- i. Carefully pour approximately 5 mL of ice cold isopropanol over a solution of DNA.
- ii. Use a glass rod is used to mix the two liquids at their interface. The chromosomal DNA that is abundant will form a viscous mass and will precipitate at the interface of both liquids. The DNA can then be collected on the rod.
- iii. Within the precipitate, there may be small fragments of DNA and some amount of degraded RNA. However these pieces of nucleic acids are too short in length and form precipitates that are less viscous, so it may not be able to collect these using a glass rod.

Spooling is therefore a practical method that partially purifies and concentrates high molecular weight DNA. This "purified" DNA can then be subjected to agarose gel electrophoresis to determine the integrity of spooled DNA (i.e., if it is intact or fragmented). Once electrophoresis is completed, analysis of the gel can determine if the DNA sample barely penetrated the gel and moved as a single band. If this is the case, then the DNA sample is primarily comprised of large pieces of host DNA.

Smearing patterns that move ahead of the main band correlate to the amount of degradation.

9. **Support matrices:** A support matrix can consist of various compounds such as paper, cellulose acetate, starch gel, agarose or polyacrylamide gel. The matrix is the material that will support the DNA or protein to be analysed. The purpose of using any matrix in gel electrophoresis is to prevent convective mixing that can be caused by heating. These support matrices can be stained using safe dyes such as methylene blue, (but the resolution may not be sufficiently distinct to allow for distinguishing between the matrix and the separated bands of DNA). Stained matrices can also be stored for future analysis. In research labs at the tertiary level, ethidium bromide is often used to stain separated DNA fragments. However precautions **must** be taken when using this dye and are discussed under the section of this article that focuses on Safety measures.

Two of the most commonly used support matrices are agarose gels (a natural polymer) and polyacrylamide gels (a synthetic polymer). They are used to separate molecules by size, since both these gels are porous in nature. A porous gel merely serves as a sieve, whereby larger molecules are restrained and smaller ones can migrate freely on the gel. It is easier to handle dilute agarose gels which are generally more rigid than polyacrylamide of the same concentration. Agarose gel is often used to separate larger macromolecules such as nucleic acids, large proteins

and protein complexes. Polyacrylamide is often used to separate most proteins and small oligonucleotides that require a small gel pore size to impede these molecules.

IMPORTANT FACTORS THAT AFFECT THE MOBILITY OF DNA FRAGMENTS IN AGAROSE GEL

MATRICES

In order to optimize the separation of different sizes of DNA fragments, careful attention must be paid to several factors. These are as follows:

1. The concentration of agarose in the gel (usually 0.8% w/v agarose on a tris-borate-EDTA buffer)
2. The voltage applied to the electrophoresis chamber
3. The correct type of electrophoresis buffers (e.g., tris buffer or the loading buffer)
4. The effects of methylene blue on DNA

It is important to use the correct concentration of agarose gel, so one can resolve the various sizes of DNA fragments. The higher the concentration of agarose gel used, the greater the facilitation of the separation of small DNA fragments (usually measured in base pairs - bp or kilobase pairs - kb). Conversely, the lower the concentration of agarose gels, the greater the resolution of larger DNA fragments. Agarose gels with low percentages are usually more fragile.

It is very important that the correct voltage be used in agarose gel electrophoresis of DNA. Larger fragments of DNA will migrate faster than smaller fragments if there is an

increase in the voltage applied to the gel, but generally speaking when the correct voltage is used, smaller fragments will migrate faster. Voltages of 9V are recommended for high school gel electrophoresis activities. Higher voltages may result in the disintegration of the agarose matrix. Applying lower voltages may result in incomplete separation of the bands of DNA. If the electrical field is not even across the gel, then molecules of the same size may migrate to different positions on the gel. This is termed the "edge effect" (http://www.uta.edu/biology/payne/3445/agarose_gel_electrophoresis.htm).

Using the correct concentration of buffers in agarose gel electrophoresis will ensure an optimum pH range and concentration of ions for conductivity. It also ensures that the integrity of the gel matrix will remain during electrophoresis and that the DNA will move through the gel once it has been subjected to an electric current. High concentrations of buffers will cause an exothermic reaction within the gel and may cause the gel to disintegrate. It is also important not to substitute water for the buffer, since conductivity will not be ensured, and the DNA fragments will not migrate across the agarose gel. The concentration of salts in the buffer may also affect DNA that has been digested using restriction endonucleases. This is sometimes termed the "salt effect" (http://www.uta.edu/biology/payne/3445/agarose_gel_electrophoresis.htm)

Both *in vivo* and *in vitro* studies show that there are effects of methylene blue on DNA (<http://mbcr.bcm.tmc.edu/pburch.html>). However, reasonable success can be achieved in staining DNA (isolated from plant cells) with methylene blue, which is

considered to be a safe stain that can be used in high school gel electrophoresis experiments.

The results obtained using methylene blue to stain separated bands of DNA may be influenced by the age of the stain. As a precaution, all stains are best stored in dark glass bottles or kept in the dark.

PROTOCOL IN ASSEMBLING GEL APPARATUS AND IN "RUNNING" A GEL: AGAROSE GEL

ELECTROPHORESIS

MATERIALS NEEDED

1. Gloves (NITRILE, LATEX or VINYL)
2. Agarose powder or pre-cast agarose gels
3. Loading dye (e.g., bromophenol blue)
4. TAE or TBE Electrophoresis Buffer (20X stock and 1X stock)
5. Gel electrophoresis separation trays or chambers with safety lid, solid platinum wire and electrodes
6. Gel casting tray or holder (Plexiglas) with end dams and well-combs.
7. Power supply (e.g., batteries - 9 volts or a variable power supply with the ability to run 3 chambers simultaneously)
8. 10 μ L micropipette with disposable tips.
9. DNA to be separated (usually generated from spooling)
10. DNA ladders or "standards"
11. Marker dyes (for the DNA simulation experiment) e.g. bromophenol blue, janus green, Orange G, Safranin O, Xylene Cyanol

The methodology involved in gel electrophoresis is fairly simple, but it requires a precise set up in order to obtain good separation of the macromolecule being used. If fragments of DNA are to be used, then the most commonly used ingredients in making a matrix to electrophorese the DNA mixture are either agarose or starch gels, commonly sold in powdered form.

Agarose gel is a purified polysaccharide polymer that is isolated from seaweed (e.g., Phyla Rhodophyta or Phaeophyta). Molecules of agarose are extremely water-soluble due to the large numbers of hydroxyl groups attached to this macromolecule. Solutions containing agarose tend to be of low melting points. Agarose gels are considered to be superior to starch gels because of their consistency and smoothness of the gel matrix after its preparation, analysis and storage. When heated to 100°C it melts but resolidifies when cooled below 45°C. It is during the solidification process that agarose forms a matrix of microscopic pores. The size of the pores formed is dependent on the concentration of agarose used. The most widely used concentrations will vary from 0.5% to 2.0%. The lower the concentration of agarose gel used, the larger the pore size developed within the gel during solidification.

In many high school labs, carrying out gel electrophoresis will involve the use of Agarose gel matrices. Agarose powder is usually mixed in a buffer solution, usually Tris Borate EDTA, commonly referred to as TBE buffer³. This solution is heated until the agarose powder has dissolved. The hot agarose solution is usually poured into a Plexiglas holder

³ The protocol for making up this buffer is given in Appendix 1.

(tray) and is allowed to solidify onto that support. During the cooling process, the hot agarose solution becomes polymerized into a semi-solid matrix or gel. The gel becomes translucent in 10-15 minutes and this indicates readiness for use in separating DNA or for use in a simulated DNA experiment using a mixture of marker dyes that can be separated based on their molecular weight.

After the gel has been poured, cooled and has solidified, the Plexiglas tray (gel casting chamber) containing the gel is placed in an electrophoresis chamber. This chamber is filled with buffer to cover the gel to a depth of usually about 1-2 cm in depth. This important step is to ensure that the electric current should flow from the positive pole to the negative pole at opposite ends of the gel, thus promoting separation of the macromolecule sample.

A well comb is used to imprint a series of small wells at one end of the gel. The well comb is inserted before the gel is poured. If the well comb is inserted after the gel has cooled, the gel will crack if the agarose is above a certain concentration (e.g., 0.8 % w/v of agarose in Tris EDTA buffer). The wells function as reservoirs for holding the DNA sample. These wells are usually equidistant in spacing, and each reservoir should be of the same volume. These factors are important in minimizing variability when the macromolecule mixture is loaded into each well. The DNA ladders or "standards" should be loaded into wells either on the right or left of the slab of gel so that the macromolecules in the "test" well can be easily compared.

The samples of DNA fragments to be electrophoresed, can be mixed with a loading buffer containing a tracking dye usually bromophenol blue, that will enable the instructor to track the samples as they migrate from each well. The solution of loading buffer should also contain glycerol⁴ or sucrose in order to ensure that the mixture is heavy enough to sink to the bottom of each well. Loading the wells with the mixture of macromolecules should be carried out using a micropipette to ensure that a constant volume of test mixture is loaded into each well. **It is important to change the tip of the micropipette after loading each standard to prevent contamination of the test sample.**

An electric field used in gel electrophoresis is normally provided by a variable power supply. Each electrode from the power supply should be attached to the appropriate terminals on the gel electrophoresis apparatus chamber (containing the gel material and the test mixture of macromolecules and the standards of known molecular size). The anode (positive) connected to the electrophoresis chamber will usually be coloured red and the cathode (negative) black. DNA will usually migrate towards the anode, due to the negative charges conferred by the phosphate backbone. Before the circuit is closed, place a safety cover over the electrophoresis tray. The electric current is usually turned off after a run time of 10 - 40 minutes depending on the amount of sample mixture. For example, a 5 μ L of sample may require a run time of about 40 minutes using a voltage of nine volts, whereas a 15 μ L sample may require a run time of approximately 1.25 hours. The DNA fragments should be separated since they migrate according to their molecular size. Confirmation that the electric current is flowing through the gel is by observing bubbles coming off the electrodes.

⁴ Glycerol and sucrose has a density greater than water

Once the loading dye has reached the top of the gel, the electrophoresis procedure is deemed complete. The next stage involves applying a staining protocol, usually using a safe methylene-based dye in high school classrooms. Methylene blue ($C_{16}H_{18}N_3SCl \cdot 3H_2O$), is a basic aniline dye. There are properties of methylene blue that allow it to stain DNA effectively. One such property is its photochemical nature (i.e., it can be activated by light to an excited state). This reaction in turn activates oxygen to yield oxidizing radicals. These oxidizing radicals can generate cross-linking of amino acid residues on proteins (Schneider *et. al.*, 1998). Methylene blue can also bind loosely with the phosphate backbone of DNA to some degree, thus producing visible bands on the gels.

Thiazin dyes in aqueous solution (usually dissolved in the running buffer at pH 7.5) are applied to the gel after it has been run. Since the entire gel may be heavily stained with the characteristic blue colour, destaining with dilute acetic acid or 0.2 M sodium acetate buffer (pH 4.7) is often recommended.

If the gel is allowed to stain for five minutes, the bands of separated DNA are usually quite prominent. The final step would include plating the gel in a tray of water and allowing it to "destain" for approximately 60 minutes⁵. The gel is then removed from the tray and can be viewed immediately using a UV light box. This is done since methylene blue stains fade rapidly after it is used.

ALTERNATIVE DYES TO USING ETHIDIUM BROMIDE FOR STAINING DNA IN GEL ELECTROPHORESIS

Any stain (e.g. ethidium bromide) that intercalates with DNA should be treated as a potential mutagen, teratogen and carcinogen and its use should be avoided. Alternatively, In high school classrooms, methylene blue can be used to stain electrophoresed gels. However, methylene blue may stain the entire gel, thus obscuring the separated bands of DNA and hence the resolution between the various molecular sizes of the DNA fragments may not be precise. Some research procedures where plant DNA is used report separation of fragments that are precise. These research labs have reported success in using commercially prepared methylene-based stains (e.g., Carolina Blu) to stain DNA. The protocol for using methylene blue (as suggested on the URL Web Site <http://wheat.pw.usda.gov/~lazo/methods/lazo/met1.html>) as an effective DNA stain is outlined in Appendix 2.

Adkins and Burmeister (1996) also have also identified other dyes that may be useful for visualising DNA. They suggest a dye containing a mixture of Nile blue sulphate and methylene blue. While the specific mode of action is unknown for this dye, Nile blue sulphate is thought to intercalate within the DNA double helix. Therefore caution must be exercised if using Nile blue sulphate or avoided altogether until there is pertinent information on the exact mode of action of Nile blue sulphate.

⁵ Depending on the degree of staining, prolonged destaining may be necessary, sometimes over a 24 hour period.

As an alternative procedure, students can electrophorese "known" marker dyes such as bromophenol blue, janus green, Orange G, Safranin O and Xylene Cyanol and a "test" mixture of dyes instead of a sample of DNA. This dye mixture will separate into bands based on the sizes of the particles and can be identified against the "known" marker dyes. It can also be inferred that these dye particles are also separated based on their charges. This simulation exercise is intended to give students hands on practice in a gel electrophoresis experiment before actually using a sample of DNA. It also circumvents the use of ethidium bromide in high school classrooms. The separation of the dye mixture will remain distinct only for a few hours, before integrity of the separated bands is lost. Teachers and instructors can also supplement the simulation activity by referencing colour photographs of the gels (viewed using a UV light box) to show the separated DNA fragments by gel electrophoresis using ethidium bromide (Giuseppe *et. al.*, 2002). The DNA bands in these photographs are usually discrete.

SAFETY PROCEDURES TO ADHERE TO WHEN PERFORMING THE GEL ELECTROPHORESIS PROTOCOL

In executing any laboratory activity, there are inherent rules and safety procedures that are mandatory in order to promote efficiency and above all safety for all involved. Reviewing standard lab safety with students by having a peer-teaching session on laboratory safety during the pre-lab talk is a useful method for adolescent students. A sample list of these rules is provided in Appendix 3.

In addition to these standard rules, there are additional and specific safety features to follow when performing gel electrophoresis. The following sections will explore specific laboratory rules that students may not have been met before in other strands of biology or in any science course. **It is unlikely that experiments on DNA separation by gel electrophoresis using ethidium bromide will be carried out in high school experiments. However, due diligence is fundamental if any harmful chemicals are to be used at any level of learning. Appendix 4 provides useful information on the containment of ethidium bromide so that instructors and teachers can discuss them with students during post-lab analysis. It is extremely relevant and pertinent to the concepts being discussed in the Biotechnological tools and Techniques section in the Nelson Biology 12 text book that is widely utilized in classroom across Ontario.**

The section below lists important safety rules directly related to the protocol of agarose gel electrophoresis.

1. Donning gloves when handling chemicals is not only a standard safety rule, but it also ensures that nucleases present on the skin on finger tips will not degrade DNA.
2. Before the electrophoresis procedure begins, it is suggested that the ends of the gel tray be secured with a small amount of tape on the underside.

3. Since buffers and other chemicals will be used in the electrophoresis chamber, the electrophoresis tray should be placed on a sheet of plastic or on a non-reactive surface, in case of spillage.
4. If a microwave is being used to heat the agarose gel in water, then add the appropriate amount of agarose to approximately 50 mL of water in an Erlenmeyer flask. Heat the mixture in the microwave on high setting for approximately 90 seconds until the mixture begins to boil. Exercise caution in removing the hot Erlenmeyer flask from the microwave by using tongs or wire padded gloves (e.g., the type used to remove hot glassware from autoclave machines).
5. Before closing the circuit in order to separate the DNA mixture, ensure that the electrophoresis chamber is tray is covered to prevent electric shocks.
6. The use of ethidium bromide (Et Br) dye to stain DNA requires strict guidelines. Under no circumstances should Et Br be used without gloves. Wearing two pairs of gloves ("double gloving") is an extra measure that can provide more rigorous personal safety. The concentration of Et Br used in gel electrophoresis is normally 10 mg/mL solution (in water). Buying a commercial preparation of Et Br can reduce personal exposure. Ethidium Bromide is a known carcinogen and mutagen that may be absorbed through the skin. Its toxicological properties have not been fully investigated. The MSDS on Et Br should be consulted.

7. **IT IS IMPERATIVE that goggles or face shields and protective clothing such as a lab coat are used during this procedure.** When viewing the stained gels containing the separated DNA, they should be placed on the trans-illuminator. The clear plastic shield is then closed and then the UV lamp is turned on. the transilluminator emits short wave UV light. This band of UV light which will damage skin and eyes, if there is prolonged exposure.

CONCLUSION

The information provided in this article is intended for educators and instructors who are planning, implementing and disseminating lessons on various aspects of molecular genetics and biotechnology. Whereas we must concentrate on safe practices when carrying out gel electrophoresis in high school activities, we must also be cognizant of disseminating useful information on the techniques that support experimental protocols in electrophoresis. The use of potentially toxic compounds at the high school level should be minimized, but due diligence must be highlighted in any experimental activity. The information on the use of ethidium bromide is included to provide educators at the high school level with important information on safety practices surrounding the use of toxic compounds. This compound among many other ones may be used in demonstrations for their Grade 12 students after their entry into University programs such as in a first year Introductory Biochemistry course or a Molecular Genetics course. It is therefore incumbent upon educators to inform students of the chemistry of these compounds to facilitate responsible lab safety practices. Since Biotechnology is one of the fastest growing fields in Biology, we need to prepare our

students for further research within this field by providing them with the most fundamental tool in learning - how to be current in research through the avenue of critical thinking.

REFERENCES

1. Adams, R.L.P., J.T. Knowler and D.P. Leader (1992). Biochemistry of the Nucleic Acids. Eleventh Edition. New York: Chapman and Hall.
2. Acquah, G. Ph.D. (1992) Practical Electrophoresis for Genetic Research. by Dioscorides Press. Portland, Oregon. p. 19, 49.
3. Ausubel, F.M., Brent, R, Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and K. Struhl, (2000). Current Protocols in Molecular Biology. John Wiley & Sons, Inc., p.255 - 257.
4. Campbell, M. K. (1991). Instructor's Manual with Test Questions for Biochemistry. Saunders College Publishing, a division of Holt, Rinehart and Winston p. 161.
5. Glick, B.R. and J.J. Pasternak (1994). Molecular Biotechnology: Principles and Applications of Recombinant DNA. Washington, DC: American Society for Microbiology.
6. Giuseppe M.D., A. Vavitsas, B. Ritter, D. Fraser, A. Arora and B. Lisser., (2002). Biology 12. Copyright Nelson, a division of Thomson Canada Limited. p.278-284.
7. Kornberg, A. and T. Baker (1992). DNA Replication. Second Edition (New York: W.H. Freeman.
8. Matthews, C.K. and K.E. Van Holde (1996). Biochemistry. Second edition. P 87-131. The Benjamin/Cummings Publishing Company.
9. Singer, M. and P. Berg (1991). Genes and genomes: A changing perspective. Mill Valley, CA: University Science Books.

10.Solomon, E.P., L.R. Berg and D.W. Martin (1999). Biology. Fifth edition; Saunders College Publishing; Harcourt brace College Publishers. p. 311 and 312.

JOURNAL ARTICLES

Nile blue sulphate (<http://www-personal.umich.edu/~steviema/blueDNA.html>)

Adkins, S. and Burmeister, M. (1996) Visualization of DNA in agarose gels as migrating colored bands: Applications for preparative gels and educational demonstrations *Analytical Biochemistry* 240 (1) 17-23.

Flores, N. *et al* (1992) Recovery of DNA from agarose gels stained with methylene blue. *Biotechniques* 13, 203-205.

J. E. Schneider, Jr., T. Tabatabaie, R. H. S. L. Mardt, X. Nguyen, Q. Pye, R. A. Floyd, **"Potential Mechanisms of Photodynamic Inactivation of Virus by Methylene Blue I. RNA-Protein Crosslinks and Other Oxidative Lesions in Q \beta Bacteriophage,"** *Photochem Photobiol*, **67**, 350 (1998).

Sinclair, B. **Safe and sensitive new stains replace ethidium bromide for routine nucleic acid detection.** *The Scientist* 14[8]:31, Apr. 17, 2000

Yung-Sharp, D. and Kumar, R. (1989) Protocols for the visualisation of DNA in electrophoretic gels by a safe and inexpensive alternative to ethidium bromide. *Technique* 1 (3) 183-187.

GENERAL URL REFERENCES ON MOLECULAR TECHNOLOGIES

Electrophoresis Society Glossary of Terms (acronyms and abbreviations, to be expanded) 2000, 350+ terms <http://www.aesociety.org/AESgloss.html>

Restriction Enzymes: Cleavage of DNA lab University of Illinois. (1999). Experiment 2 Gel Electrophoresis of DNA. In Molecular Biology Cyberlab, online: <Http://www.life.uluc.edu/molbio/geldigest/electro.html>

“Ecological and Evolutionary implication of Bt cotton. Measurement of a single gene difference in two cotton plants by PCR” uses 0.025% methylene blue.

http://biotech.biology.arizona.edu/labs/bt_cottonSG.html

"Biotechnology," Microsoft® Encarta® Online Encyclopedia 2003 <http://encarta.msn.com> © 1997-2003 Microsoft Corporation. All Rights Reserved.

Mitochondrial (mt) Point Mutations protocol uses up to 0.05% methylene blue and readily amplified DNA from the mitochondrial genome which is easily seen with this stain. <http://www.geneticorigins.org/geneticorigins/>

URL SITES ON SAFETY DATA ON METHYLENE BLUE

http://physchem.ox.ac.uk/MSDS/ME/methylene_blue.html

<http://www.jtbaker.com/msds/englishhtml/m4381.htm>

URL REFERENCES ON GEL ELECTROPHORESIS PROTOCOLS

Protocols on gel electrophoresis using micro and macro amounts of agarose gels - gel recipes. <http://haveylab hort.wisc.edu/protocol/gel.html>

Practical information on gel electrophoresis and important information on gel artifacts.

(http://www.uta.edu/biology/payne/3445/agarose_gel_electrophoresis.htm)

URL WEB SITES ON ALTERNATIVES TO USING ETHIDIUM BROMIDE

DNA Gel Electrophoresis Staining Alternate to Ethidium Bromide. Source: *Dolan DNA Learning Centre, Cold Spring Harbour*

<http://www.geneticorigins.org/geneticorigins/mito/recipes3.htm>

An excellent web site that compares the use of Ethidium Bromide with safer alternative DNA dyes <http://www.bioscience-explained.org/EN1.2/schollar.html>

APPENDIX 1

PROTOCOLS FOR MAKING TRIS BORATE (EDTA) TBE AND THE GEL LOADING BUFFER

10x TBE

108 g Tris base

55 g boric acid

40 ml 0.5 M EDTA, pH=8

distilled water to 1 liter

6x gel loading buffer

0.25% Bromophenol blue

0.25% Xylene cyanol FF

15% Ficoll Type 4000

120 mM EDTA

APPENDIX 2

METHYLENE BLUE DNA STAINING PROTOCOL (adapted from the URL Web Site

<http://wheat.pw.usda.gov/~lazo/methods/lazo/met1.html>

Protocol:

1. Load 2-5X the amount of DNA that would give bands of moderate intensity on an ethidium bromide stained gel. Typically this is something on the order of 0.5-2.5 μg of a 1kb fragment on a 30 mL 1% mini gel. These numbers are estimates so results may vary.
2. Run the gel normally and then place in a 0.002% methylene blue (w/v, Sigma M-4159) solution in 0.1X TAE (0.004M Tris 0.0001 M EDTA) for 1-4 hours at room temp (22°C) or overnight at 4 °C.
3. If destaining is needed to increase the visibility of the bands of DNA, place the gel in 0.1X TAE with gentle agitation, changing the buffer every 30 - 60 min until you are satisfied with the degree of destaining.

APPENDIX 3

A SAMPLE OF STANDARD LABORATORY SAFETY RULES

1. Wash your hands thoroughly *before* and *after* each experiment.
2. Wear a lab coat, appropriate gloves (such as latex, vinyl or nitrile) and safety goggles as personal standard safety equipment.
3. Clean your work area (laboratory station) with a disinfectant soap solution (5% v/v) *before* and *after* each laboratory period. This standard procedure lessens the chance of contaminating gel matrices with debris.
4. Do not place *anything in your mouth* while in the laboratory. This includes pencils, food, and fingers. Learn to keep your hands away from your mouth.
5. Be responsible for your lab station. Before leaving the lab, please make sure all the electrical/ battery power supply packs are disconnected from the electrophoresis chambers.
6. If toxic compounds are used such as ethidium bromide, make sure that they are used in the fume hood if one is present, or at specifically designated stations in the laboratory or classroom. Transferring chemicals from one part of a laboratory or classroom to another part can increase the risk of spillage of these chemicals and contamination by these chemicals, and therefore should be avoided.
7. When making a dilute solution of acid, an exothermic reaction occurs. Make sure that the acid is added to the water and not the other way. Add titres of acid to a larger volume of water and swirl the solution carefully.

APPENDIX 4

CONTAINMENT AND SAFE DISPOSAL OF CHEMICALS USED IN THE GEL ELECTROPHORESIS EXPERIMENT

This section provides useful information on the safety practices that surround the use of ethidium bromide (used in low concentrations) to visually track the separated DNA within a gel electrophoresis activity. **If small amounts of Et Br is being used, it is imperative that only instructors handle the gels containing this carcinogen using all the safety practices suggested.** One major concern in carrying out agarose gel electrophoresis is how to safely contain and dispose of ethidium bromide . There are several important rules to follow to safely dispose of any material that has been in contact with Et Br.

1. Concentrated stocks of Et Br must be stored in fume hoods and then disposed of as hazardous waste. Concentrated solutions (such as 10 mg/ml stocks) require dilution to <0.5 mg/ml and prolonged incubation with hypophosphorous acid (itself very corrosive and hazardous) and sodium nitrate, which reduces the mutagenicity by ~200-fold (Sinclair, 2000).
2. Gels that have been stained using Et Br should be dried out in the fume hood and disposed of as hazardous waste once dehydrated.

- 3.** Dilute solutions (such as gel buffers containing ~0.5 µg/ml EtBr) should be decontaminated using amberlite resin or activated charcoal, as the traditional methods of neutralization with bleach result in the formation of other mutagenic compounds (Sinclair, 2000). All gel running buffers should be mixed with activated charcoal. This inert material will bind the Et Br. The bound ethidium-charcoal solid can then be filtered and disposed of as hazardous waste. It is then safe to pour the remaining decontaminated liquid down the sink.

- 4.** If there is spillage of the loading buffer or the Et Br onto laboratory surfaces, or if equipment has been in contact with Et Br, then using a 0.2 M solution of nitric acid to clean surfaces can help to decontaminate these surfaces. Soaking any apparatus and equipment overnight in the 0.2 M solution of nitric acid will permit decontamination.