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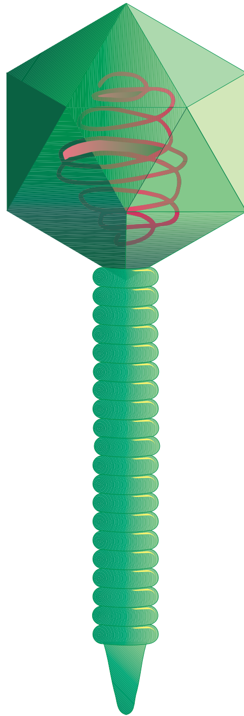
21-1008, 21-1010

Exploring Restriction Analysis and Electrophoresis of DNA

Teacher's Manual



Contents

About This Kit 3

Contacting *Carolina Biological* 3

Acknowledgements 3

Materials 4

Laboratory Safety 5

Reuse of Materials 6

Storage of Reagents 7

Hints and Tips 8

Further Resources 10

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About This Kit

The Student's Guide in this *Exploring Restriction Analysis and Electrophoresis of DNA* kit explains how to rehydrate pure DNA from bacteriophage lambda, cut it with several restriction enzymes, then separate these fragments by gel electrophoresis. Finally, the DNA fragments are stained so that they can be seen on the gels.

This safe, low cost, carefully tested and well-proven protocol will give students a flavor of the molecular biology done at the cutting edge of biotechnology today.

Contacting *Carolina Biological*

Carolina Biological welcomes comments from teachers and students using this equipment. We can be contacted at the following address:

Carolina Biological Supply Company
Biotechnology Department
2700 York Road
Burlington, NC 27215

Telephone:	1-800-227-1150
Fax:	1-910-538-6310
E-Mail:	biotech@carolina.com

Acknowledgements

The electrophoresis equipment in this kit was originally developed in 1993 by Dean Madden and John Schollar at the *National Centre for Biotechnology Education* at the University of Reading (United Kingdom).

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Materials

Supplied ready to use:

Instant Lambda DNA (long white tubes)
Instant Restriction Enzyme *Eco*RI (red tubes)
Instant Restriction Enzyme *Hin*dIII (green tubes)
Instant Restriction Enzyme *Bam*HI (blue tubes)
Empty Microtubes (colored yellow)
Electrophoresis Box
Gel Comb
Microsyringe Tips
Microsyringes
Bromophenol Blue Loading Dye (screw-topped tubes)
Foam Tray, for Holding Microtubes
Wires with Alligator Clips

In Demonstration Set Only:

0.8% Agarose Solution in TBE Buffer

In Classroom Set Only:

0.8% agarose gel, made by dissolving the agarose powder in diluted electrophoresis buffer; 1.2 g of agarose in 150 mL 1x TBE solution will give approximately 15 (0.8%) gels (each gel box will hold about 10 mL agarose).

Note: Do not dissolve the agarose in water. The easiest way to dissolve the agarose is to use a microwave oven. It is important that the concentration of agarose is correct. Don't be tempted to prepare a small volume. Make up at least 150 mL, use what you need, and keep the rest in an airtight container to prevent dessication.

The classroom set includes materials for 5 groups of students to perform the lab activity. The demonstration set includes materials for one demonstration.

Supplied, but requiring preparation:

Carbon Fiber Electrode Tissue, cut to approximately 42 × 22 mm

Electrophoresis (TBE) Buffer Solution, prepared from the 20x concentrate supplied in the kit; add 25 mL of 20x TBE to 475 mL distilled or deionized water

Note: the buffer solution may be saved and reused several times.

Carolina BLU™ concentrate DNA stain

Note: The DNA stain may be reused several times.

Materials needed, but not provided:

- 9-V Batteries, 1–3 per gel
- Distilled Water (to rehydrate the lambda DNA)
- Water Bath or Incubator set at 37° C, so that the DNA may be incubated with the restriction enzymes
- Marking Pen

Laboratory Safety

The manufacturers of this product have made every effort to check that recognized hazards have been identified and that suitable precautions are suggested. Where possible, the proposed procedures are in accordance with commonly adopted general risk assessments. If a special risk assessment may be necessary, this has been indicated. However, users should be aware that errors and omissions can be made, and that different employers and educational authorities adopt different standards. Therefore, before initiating any activity, users should always carry out their own risk assessment. In particular, any local rules issued by employers or educational authorities MUST be obeyed, regardless of whatever else is suggested by the manufacturers.

DNA and Enzymes

The naturally occurring lambda DNA and restriction enzymes provided with this kit are quite safe to use. Living organisms are not used, so there is no need to follow strict aseptic techniques. However, cleanliness is important to prevent cross-contamination and to ensure success. Spilled enzymes or DNA solution should be wiped up promptly.

DNA from a variety of sources is now becoming available. The use of DNA from certain plasmids or cloned DNA of various types may be prohibited by the safety regulations that apply to schools and colleges.

Agarose Gel

If a microwave oven is used to melt the agarose gel, ensure that the gel is placed in an unsealed container. If a microwave oven is not available, a boiling water bath or hotplate may be used instead. On a hotplate, the gel must be swirled as it melts to prevent charring. The use of a bunsen burner to melt agarose is not recommended.

Warning! Hot, molten agarose can scald and so it must be handled with care. It is advisable to let the molten agarose cool until it is comfortable to handle before pouring the gel.

Electrode Tissue
(Carbon fiber)

The carbon fiber electrode material may release small fibers, which can cause minor skin irritation if you handle the tissue a lot. It is a wise precaution to wear protective gloves if you find the tissue unpleasant to handle. However, the fibers released are too large to enter the lungs, so it is not necessary to wear a face mask. In addition, the fibers are soluble in body fluids and are completely biodegradable.

Electrical Supply

Warning! The gel electrophoresis equipment was designed to be used at low voltages (≤45 volts) with dry cell batteries. Under no circumstances should this voltage be exceeded, as the live electrical components are not isolated from the user.

Serious or lethal electrical shock could result if the equipment is connected directly to a power supply.

Note: Good results will be achieved if the gel is run between 9 and 35 volts. At higher voltages, the DNA fragments migrate faster, but they will not separate as well.

Carolina BLU™

When used as directed, *Carolina BLU*™ presents no serious safety hazard, although care should be taken to prevent splashes on the skin or eyes, e.g., wear protective gloves and safety glasses when handling the stain. Used stain can be diluted with water and washed down the drain.

TBE Buffer
(Tris-Borate-EDTA)

When diluted and used as directed, this buffer presents no serious safety hazards. Used buffer can be washed down the drain.

Loading Dye
(Bromophenol Blue/Sucrose)

When used as directed, this loading dye presents no serious safety hazards. Used loading dye can be washed down the drain.

Reuse of Materials

Electrophoresis Buffer Solution (TBE)

The TBE buffer solution can be retained and reused several times if desired. Eventually it becomes tired due to electrolysis—but at the low voltages encountered with this equipment this should not be evident for some time.

Carolina BLU™

The *Carolina BLU™* stain may also be reused. After many uses its effectiveness will be reduced as it is gradually diluted with buffer solution washed from the gel. Then, you may find it necessary to soak the gel for longer than the 4 minutes that is suggested in the student's booklet.

Agarose

Agarose is very expensive. Damaged, unused gel, e.g., from a poorly cast gel, may be re-melted and used again. However, do not attempt to reuse agarose that you've already used for electrophoresis. Agarose is not like nutrient agar— nothing will grow on it once it's been made up, so you can prepare a large batch and just use what you need. It can be re-melted and allowed to set many times (boiling is required to melt the agarose; once molten it can be maintained in that state at 55–60° C). Store prepared agarose gel in an airtight container to prevent dessication.

Microsyringe Tips and Microtubes

Both the microsyringe tips and microtubes are autoclavable, and may be washed, sterilized, and reused if wished. Although this may seem desirable to prevent waste, we don't recommend it. Even minute residual amounts of detergent on the plastic may inhibit the delicate (and expensive) restriction enzymes.

Storage of Reagents

DNA and Enzymes

The DNA and enzymes must be stored at room temperature, and be tightly sealed in the foil pouches provided. The sachet of desiccant should be kept in each foil pouch, since excess moisture can quickly lead to a reduction in the activity of the dried enzymes.

Note: It does not matter if the caps come off the enzyme tubes during storage, as long as the pouch is adequately sealed.

Buffer Solutions

Buffer concentrate should be stored at room temperature. Diluted buffer solution should be stored in a refrigerator at about 4° C.

Sometimes crystals of EDTA come out of solution in the concentrated TBE buffer. It is important that these are re-dissolved before you dilute the buffer. Do this by heating the solution, e.g., by standing the bottle in a heated water bath.

Carolina BLU™

The stain may be stored at room temperature.

Hints and Tips

Using Microsyringes

Students' ability to dispense precise volumes using the microsyringes will improve with experience. It is therefore a good idea to encourage them to practice transferring liquids (e.g., food coloring) before starting work with expensive DNA, enzymes, and other reagents. Some extra microsyringe tips are provided in the kit for this purpose.

Note: For your convenience, you may also purchase Practice Pipetting Stations from Carolina Biological Supply Co. (catalog # 21-1145).

Loading the Gel

A steady hand is required to load gels. To give students practice, gels may be cast from cheap agar (use a 1% solution) rather than agarose. When practicing, use water rather than buffer over the gel. Sufficient loading dye has been included in the kit for students to become skilled at loading into the wells.

Note: Agarose, dissolved in diluted buffer, not water, must be used for the "real" gels with DNA.

Melting Agarose

Ensure that the container used to prepare the agarose gel is clean. Tiny flecks of dust will not affect the way the gel runs, but they can prove a nuisance when you are trying to see faint bands in the gel.

For convenience, dissolve and melt the agarose using a microwave oven. Less than a minute at full power in a 940-W oven is sufficient for 100 mL of gel. The container (flask or beaker) used to hold the molten agarose must not be sealed, but covered lightly with plastic film that has been punctured with one of two small holes. Swirl the gel half way through the heating cycle to ensure that it is thoroughly mixed.

Agarose gel can be prepared using a hotplate. If this is done, the gel must be swirled as it melts, to prevent charring. Better still, stand the container of agarose in a saucepan of boiling water. The use of a bunsen burner to melt agarose is not recommended.

Once melted, the gel may be kept in a molten state by standing the container in a water bath at 60° C until the gel is needed. Take care when handling the molten gel; it will be very hot, and can scald. The gel should be allowed to cool before it is poured.

Batteries

Dry cells (the alkaline or zinc-carbon types) are suitable for use with this equipment. Several "runs" may be obtained from one battery. Rechargeable nickel-cadmium cells also give very good results, but must be completely discharged and then recharged after one or two runs to maintain their performance.

Gel Takes Too Long to Run

With 2 new or freshly charged 9-V batteries the loading dye should move about 5 mm per hour. At a lower voltage the loading dye will move more slowly.

If, after the first 20 minutes, the loading dye does not seem to have moved and bubbles are not visible at the cathode, check the electrical contacts between the batteries and the electrodes. Ensure that there is enough buffer above the gel to cover the plastic ridges at each end of the tank, but not so much that most of the current passes through the buffer solution rather than the gel. Remember than during long runs (e.g., overnight) or in a warm environment liquid may evaporate from the buffer. The tank should be covered loosely (e.g., with a plastic bag) to reduce such evaporation.

Corrosion of Alligator Clips

The alligator clip used at the anode will slowly corrode due to electrolysis. This can discolor the buffer solution and gel, but will not interfere with the electrophoresis. Eventually, you will need to replace the clip.

Viewing the Stained Gel

The bands are seen most easily by holding the gel at an angle against a well-lit surface. Several authors suggest that a yellow filter improves the contrast between stained bands and the background. While this may be true if you are taking photographs, filters can make it more difficult to spot very faint bands with the naked eye. A magnifying lens can be useful; flat fresnel lenses the size of a credit card (sold for use with atlases) are ideal. If the contrast between the background and the DNA bands is strong, the gel may be viewed using an overhead projector.

There's No DNA on the Gel

The most common cause of failure when using dried DNA arises when the DNA is not adequately rehydrated and mixed with water. A blank gel usually indicates that the DNA has not been rehydrated at all. It is essential that the DNA is thoroughly mixed with the water. To ensure that this is done, always draw the DNA solution up and down in the microsyringe tip a few times. Proper mixing is also essential when the loading dye is added to the DNA samples.

There's a Large Amount of Indistinct DNA on the Gel

This is also caused by inadequate mixing. Some of your wells are probably empty, and most of the DNA has ended up in a single well, which is consequently overloaded. Follow the instructions above to ensure that the DNA solution is thoroughly mixed.

Further Resources

Educational Papers

Micklos, D. A. and G. A. Freyer, 1989. A laboratory introduction to DNA restriction analysis. *BIOtechnology Education,* 1 (1) 16–22.

Madden, D. 1993. Batteries not included. *The Biochemist*. October/November issue, pages 21–23.

Miller, M. B.1993. DNA technology in schools: a straightforward approach, *BIOtechnology Education,* 4 (1) 15–21.

Richardson, J. 1994. DNA technology kits—a review. *SSERC Bulletin* 180, 10–21 (Spring 1994).

Miller, M. B. 1994. Practical DNA technology in school, *Journal of Biological Education*, 28 (3) 203–211.

Brown, N. 1995. Electrophoresis for the visually impaired: the modification of the lambda protocol and its use with visually impaired A-Level students, *Journal of Biological Education*, 29 (3) 166–169.

Miller, M. B. and G. A. Russell, 1996. Practical DNA technology in school— 2: Computer analysis of bacteriophage lambda base sequence, *Journal of Biological Education,* 30 (3) 176–183.

Russell, G. A. and M. B. Miller, 1996. Practical DNA technology in school— 3: Mapping and methylation, *Journal of Biological Education,* 30 (4) 289–297.

Russell, G. A. and M. B. Miller, 1997. Practical DNA technology in school— 4: Plasmids and ligase, *Journal of Biological Education,* 31 (2) 135–140.

Educational Publications

A Sourcebook of Biotechnology Activities by Alison Rasmussen and Robert Matheson 1990. National Association of Biology Teachers/North Carolina Biotechnology Center. ISBN: 0 941212 09 2.

DNA Science. A First Course in Recombinant DNA Technology by David Micklos and Greg Freyer 1990. Cold Spring Harbor Laboratory Press/Carolina Biological Supply Company. ISBN: 0 89278 411 3.

Experimental Gene Technology in Education by H. Agensen, et al. 1992. English edition. Nucleus Forlag ApS, Studsgade 28, 8000 Århus C, Denmark. ISBN: 0 06 273099 1.

Laboratory DNA Science by Mark Bloom, David Micklos and Greg Freyer 1996. Benjamin/Cummings Publishing Co., Inc. ISBN: 0 805330402.

Recombinant DNA and Biotechnology: A Guide for Students by Helen Kreuzer and Adrianne Massey 1996. American Society for Microbiology Press. ISBN: 1 55581 110 8C.

Safety

Horn, T.M. 1992. *Working with DNA and Bacteria in a Pre-College Science Classroom*. National Association of Biology Teachers.

Web Sites

Carolina Biological Supply Company (USA)

http://www.carolina.com

National Centre for Biotechnology Education (UK)

http://www.ncbe.reading.ac.uk

Access Excellence (USA)

http://www.gene.com/ae/

Advanced Placement Biology (USA)

http://www.apbio.biosci.uga.edu/

Cold Spring Harbor Laboratory DNA Learning Center (USA)

http://darwin.cshl.org/

European Initiative for Biotechnology Education (UK)

http://www.eibe.reading.ac.uk:8001

Genetics Education Network (USA)

http://www.phys.ksu.edu/gene/index.html

National Association of Biology Teachers (USA)

http://www.nabt.org/

To order call:
1-800-334-5551 (US and Canada)
336-584-0381 (International)

For technical help call:
1-800-227-1150

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