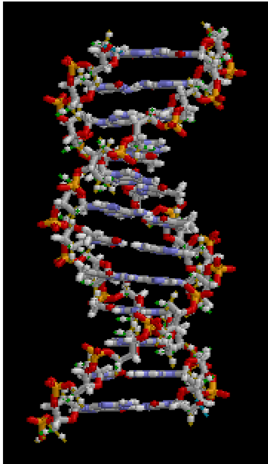

Fralin Life Sciences Institute

DNA Biotechnology Kit

INFORMATION MANUAL

Now celebrating twenty years assisting science educators!

Kristi DeCourcy
Fralin Life Science Institute
Virginia Tech
August 2013

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WHAT'S NEW?

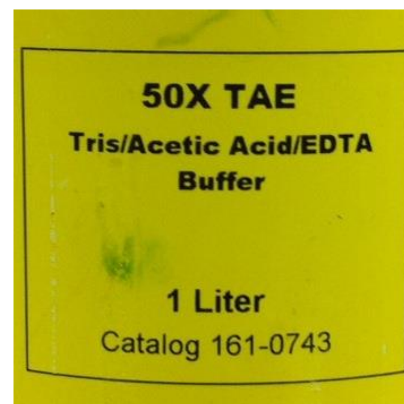
Our twentieth year!

Believe it or not, the 2013-2014 academic year will be the 20th year for the Biotech-in-a-Box program. We started with a single DNA kit (and eight borrowers, all retired now!) in 1994. Last year, the DNA kits were borrowed by 133 teachers and used with over 9,000 students! Overall, the program now offers 6 different kits and has provided over 230,000 lab experiences to Virginia students in the past 19 years.

Updated manual!

There are only a few changes, but an updated look!

Back to TAE buffer!

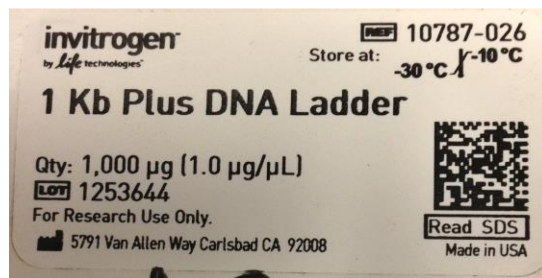


We're switching back to TAE buffer. The gels will be prepared with 1x buffer, as always, but the concentration for running buffer will be 0.25x. This concentration will allow the gels to be run at 200 volts. This is slightly below the voltage that we could use with SB buffer, but the gels will still run in under 20 minutes. Please follow the updated directions (page 27) in the manual (and I'll include a brightly colored flyer in the kit).

No books/DVD

Since shipping is our highest single cost, we're dropping the two (heavy!) books from the kit. There are many more web resources available now than there were 20 years ago, so we hope you won't miss them too much. If there is an activity in one of the books that you'd like for me to copy and send to you, please let me know.

Optional DNA ladder



The DNA ladder is now an optional component of the kit. Some teachers are using scenarios and not graphing the ladder, and omitting the ladder from those loans will save us a lot of money. (It cost over $2,300 last year just for the DNA ladder.) DNA samples will be sent automatically, but teachers will have to confirm on the kit request form that they want the ladder and for how many classes.

Light box issue!

As many of you know, the light boxes that we've included in the kit since the beginning are no longer available, and the only substitutes that I've been able to find are over $100 each, beyond what we can afford. We will continue to include light boxes in the kits as long as we have them, but there may not be the full number you expect.

Acknowledgements

Large portions of this manual (calendar, several scenarios, and SOL material, in particular) were written by Erin Dolan, currently at the University of Georgia.

INTRODUCTION

The DNA Biotechnology Kit is provided by the Fralin Life Science Institute at Virginia Tech. The loan period for the equipment is two weeks, though longer loans may be arranged for multiple teachers at the same school. Please be certain that you return the equipment on time, but if you need an extension, please contact us. We will accommodate you if possible. We would much rather extend the loan period than have the equipment return to us unused.

Please read the directions in this manual, in particular the directions on how to use the equipment and the directions for packing the equipment for shipment. Thus far, the loaner program has not lost much equipment to breakage/misuse, and we hope to keep it that way.

This manual has been written to provide background scientific information, student activities, scenarios that can be used to make the activities more stimulating for your students, thought questions that can be raised during the activity, and detailed instructions on the use of the equipment (probably too much detail in many cases).

Since there is tremendous variation around the state in class period length, the experiments are not written in lesson format; you'll have to adapt them to your specific needs. See the Calendar of Activities below (page 6) for some suggestions on timing. The table includes timing for different class lengths. Feel free to call Kristi if you have questions.

The labs require some preparation, such as aliquoting the reagents. The reagents that need to be aliquoted are listed at the beginning of each lab.

At this time, the Institute is providing the materials needed to analyze DNA samples by gel electrophoresis (with optional mapping of a DNA ladder). There are other experiments that may be done while you have the loaner equipment. For example, there are many samples available through biological supply companies (e.g., Carolina Biological Supply Company, Bio-Rad, Edvotek, or Ward's Biology) that could be analyzed using the gel electrophoresis equipment.

Please read the "**Important Notes**" on page 7! This is where problem areas of which all borrowers need to be aware are listed.

We welcome all comments and suggestions, and apologize for any typos and errors. Let us know what else you would like to see in the manual.

DNA BIOTECHNOLOGY KIT CONTENTS

The contents of the DNA Biotechnology Kit are listed in the table below. Contents many vary slightly, depending on class size. For a “normal” full size class, the kit will contain equipment for 8 student work stations. The consumables are shipped according to the number of classes that will be using the equipment. For example, if there are 2 classes using the kit, there will be enough DNA, buffer, agarose, etc., for 16 student groups (2 classes of 8 groups).

The consumables will be included within the kit, since all items are stable for shipment at room temperature. It will be necessary for the instructor to use the Fisher micropipettor to aliquot the reagents for the students. Instructions for aliquoting are included in the procedures section of this manual, and colored microcentrifuge tubes have been provided (for color-coding reagents). A “cushion” has been added to the volumes shown below, so there should be plenty for aliquoting.

Description	Quantity
Microcentrifuge	1
Electrophoresis power supplies	4
Electrophoresis chambers	8
Mini-Pro light boxes	4–8 (see note on page 3)
Thermal grippers	2
Fisher Micropipettor (5-40 µl)	1
Graduated cylinder, 100 ml	1
Graduated cylinder, 10 ml	1
10-µl fixed volume pipettors	8
Yellow tips	1 bag
Microcentrifuge tube racks	8
Microcentrifuge tubes (1.5 ml)	1 pack
Colored microcentrifuge tubes (1.5 ml)	1 pack
Practice pipetting gels	1 pack
Non-mercury thermometer	1
Plastic boxes	8
Sharpie and label tape	1

Reagent	Amount per group	Amount per class	Storage
DNA samples	10 µl of each	100 µl	refrigerator
1 kb Plus DNA ladder (optional)	10 µl	100 µl	freezer
Agarose	0.27 g	5 g	room temp
50x TAE buffer	2 ml	25 ml	room temp
Practice pipetting dye	100 µl	5 ml	room temp
50X methylene blue (reuseable)	1 ml	8 ml	room temp

Calendar of Activities: Electrophoresis with Pre-cut DNA

Class schedule	Optional pre-lab	Day 1	Day 2	Day 3
45-50 minute periods with no blocks	Do as a pre-lab, instead of on Days 1 and 2 • Strawberry DNA extraction • Practice pipetting	• Optional strawberry DNA extraction (30 min) • Pour gels (15 min), practice pipetting while gel hardens	• Load and run (45 min), optional strawberry DNA extraction while gel running	• Stain (15 min) • Destain (15 min) • Analysis • Wrap-up
45-50 minute periods plus one block	Regular day Do as a pre-lab on a regular day, instead of Day 1 • Strawberry DNA extraction • Practice pipetting	Block day • Pour gels (15 min), practice pipetting while gel hardens • Load and run (45 min), optional strawberry DNA extraction while gel running • Stain (15 min) • Destain (15 min)	Regular day • Analysis • Wrap-up	
All blocks (90-120 minute)		Entire block • Pour gels (15 min), practice pipetting while gel hardens • Load and run (45 min), optional strawberry DNA extraction while gel running • Stain (15 min) • Destain (15 min)	Part of block • Analysis • Wrap-up	

Optional stopping points:

1. After pour gel but before loading. Store gel in plastic wrap or sealed sandwich bag with a very small amount of 1x TAE buffer in the refrigerator. The gel will keep for several days.
2. After running gel but before staining. Store gel in plastic wrap or sealed sandwich bag with a very small amount of 1x TAE in the refrigerator. The gel will keep overnight.
3. After staining gel while destaining. Store gel just barely covered with water in dish (e.g., weigh boat, plastic container, etc.) covered with plastic wrap. The gel will keep overnight.
4. After destaining gel. Store gel in plastic wrap or sealed sandwich bag in the refrigerator (no need to add liquid, the gel will be wet enough from the destaining process). The gel will keep for several days.

IMPORTANT NOTES (PLEASE READ!!)

1. Our primary equipment losses are due to user carelessness. First, it is very important that the agarose solution be cooled to 50-60˚C before it is poured into the gel trays. A thermometer is included with the kit, so it is very easy to check the temperature before the agarose is poured. *Please* check the agarose solution temperature before you pour it.

2. The reason that the 0.25x TAE running buffer (like the 1x SB buffer previously) allows us to run the gels at a much higher voltage (and hence, much more quickly) is that the buffer has a very low molarity. So, it is **essential** that the 50x TAE buffer stock be correctly diluted to the 0.25x concentration. If the 50x stock buffer is not diluted to 0.25x, the ionic strength of the running buffer is far too high. High ionic strength will make the buffer solution overheat (boil!) when the current is applied, *and* your gel will melt! **Note: you do not *have* to run gels at 200 volts; they will run just fine at any voltage <200 volts. Remember, prepare the gels with 1x TAE and use 0.25x TAE as running buffer.**

3. The coating on the metal casting gates in the Bio-Rad gel boxes can be damaged if the casting tray is removed from the gel box without first removing the casting gates. The gates can also be damaged by rough handling. Please make sure that your students are aware of this. Also, please remember to remove the casting gates *before* you start electrophoresis.

4. Please help up keep our expendables costs down by returning unused material. On the checklist, if something is shaded in the "repacked" column, it means that it is an expendable item. It is not expected that you will return all of these items, but please return any that are unused. **Do not mix** used items with unused items!
   On the checklist, if something is not shaded in the "repacked" column, that means that it is **not** an expendable item, and **all** should be returned, barring a problem, (e.g., if your dog eats a tube rack).

5. **Cable ties!** Believe it or not, there is a right way and a wrong way to secure the trunks with the cable ties. We've had trunks come back totally unsecured, and this was probably due to the cable ties being put on incorrectly. Look at the end you put the tab through. The tab should be put in from the side that is smooth with the tie, not the end that sticks out. If it is done the wrong way, the cable tie will open when you pull on it. Please test the cable tie by pulling to be sure that you've done it correctly.

6. ***Please*** keep to the schedule for return shipping. We have only a 2-day turn-around to get the kit out to the next teacher, and if the kit is not back on time, it is the next teacher who will suffer for it. (See note on page 4 about requesting a loan extension.)

7. Although we know that you will use the equipment with care, we recognize that some equipment may be damaged accidentally. Please do not try to repair the equipment; we would rather do the repair work here. Either call or put a note in the kit (see above) if there is an equipment problem. If something is missing from the shipment or arrives damaged, please call immediately.

Safety note: Please follow the guidelines established by your school district for liquid handling, especially in terms of safety glasses.

DIGESTING DNA WITH RESTRICTION ENZYMES: BACKGROUND INFORMATION

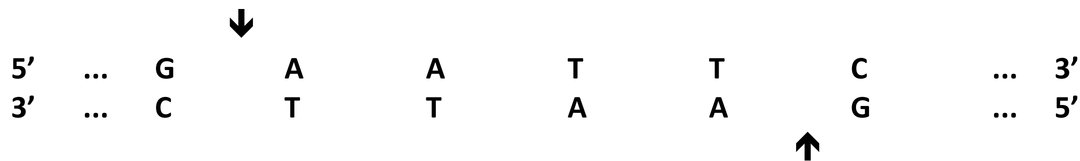
In the following experiments, students will either:

1) digest plasmid DNA with restriction enzymes and analyze the results (DNA and restriction enzymes will be provided).

or

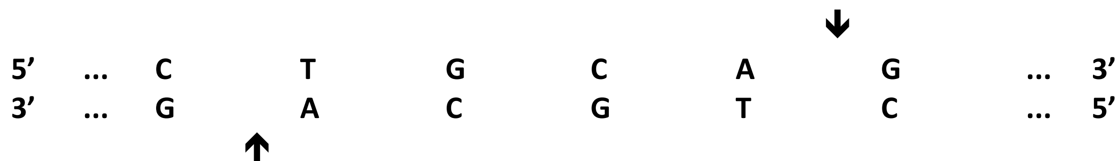
2) analyze the results of restriction enzyme digests of plasmid DNA (predigested DNA will be provided).

Restriction enzymes are enzymes that cleave double stranded DNA at specific nucleotide sequences. They are also called restriction endonucleases because they cleave at internal (endo-) locations in DNA, as opposed to exonucleases that cleave near the end of DNA molecules. The specific sequences where restriction enzymes cleave are the recognition sequences for the enzymes. For example, the recognition site for EcoRI is:



The EcoRI recognition sequence is symmetric. Symmetry in recognition sequence is a characteristic of many restriction enzymes. The enzyme cleaves at the same place on each strand, leaving a 3'-hydroxyl on one strand and a 5'-phosphate on the other. For EcoRI, this cut leaves overhanging ends (called sticky ends). Other enzymes may leave blunt ends, for example, if an enzyme were to cut between the A and the T in the recognition sequence shown above.

PstI also leaves overhanging ends. The PstI recognition sequence is:



Restriction enzymes are microbial products, and their names are derived from the names of the organisms in which they were found. For example, EcoRI was found in *Escherichia coli* (*E. coli*) and PstI was isolated from *Providencia stuartii*. EcoRI was among the first restriction enzyme purified from bacteria over 20 years ago. There are currently about 2500 restriction enzymes known, with 200 different recognition sequences.

It is believed that the native function of restriction enzymes was to digest foreign DNA, in other words, to protect the microorganism from invading viral DNA. They are now used in a variety of ways in the lab. One of the uses is construction of a map of DNA.

Another laboratory use is production of specific fragments of DNA, for example, a sequence coding for a protein of interest.

Then this particular fragment can be manipulated, e.g., cloned into a expression vector so that large amounts of the protein can be produced.

Using restriction enzymes to map DNA

The information gathered from performing multiple restriction digests on a piece of DNA can be used to make a map of the DNA. A map of a plasmid is a graphic representation showing where the restriction sites (enzyme recognition sites) are in relation to each other. To map DNA, it must be digested with several restriction enzymes (2 or more) and by the same enzymes in a multiple digest. To prepare the samples used in this experiment, the plasmid DNA, pHokie, was digested in three ways: by EcoRI, by PstI, and by a mixture of the two enzymes. By analyzing the digests using agarose gel electrophoresis, one can estimate the size of each fragment. From this information, you can map the DNA; in other words, you can locate where the restriction sites (enzyme recognition sites) are on a drawing of the plasmid.

DNA SEQUENCING

DNA sequencing has become routine. The Human Genome Project was completed a decade ago, and the complete genomes of many other organisms have also been sequenced, including most of the common model organisms used in research. Diseases have been traced to specific alterations in DNA sequence, and genetic testing is also done routinely. Sequencing means just what it says: to determine the sequence of a strand of DNA, i.e., the order of the A's, G's, C's, and T's. This used to be very labor-intensive, but advances in technology have automated the process and made it possible to sequence entire genomes in much less time (and much more cheaply) than was ever believed possible.

The chemistry has not changed tremendously, though the technology has. To determine the sequence of a piece of DNA, the DNA must be synthesized *in vitro*, in 4 different reaction mixtures. Each mixture contains an altered nucleotide. For example, the first mix contains all 4 normal nucleotides plus an altered A. The second mix would contain an altered G, etc. The altered nucleotides are called dideoxynucleotides and when one is incorporated in the newly-synthesized strand, the synthesis stops. That's where this method gets its name: the chain termination method, or Sanger sequencing (after its inventor). (An animation of Sanger sequencing may be seen at http://www.dnaftb.org/23/animation.html)

If you synthesize enough of the DNA molecule of interest, with adequate amounts of the normal and altered nucleotides, you should end with samples containing all possible lengths of the DNA molecule of interest. In the A tube, there should be molecules that end at every A and the in G tube, molecules that end at every G, etc. So each tube will have many DNA molecules of every possible length- from very few nucleotides to the full length DNA molecule (if no altered nucleotides were incorporated at all).

The DNA molecules from each of the four reactions are then separated by size. Previously the separation was performed on a sequencing gel, but currently the molecules are separated in microcapillary tubes. By either method, it is possible to separate DNA molecules that differ in size by only one nucleotide.

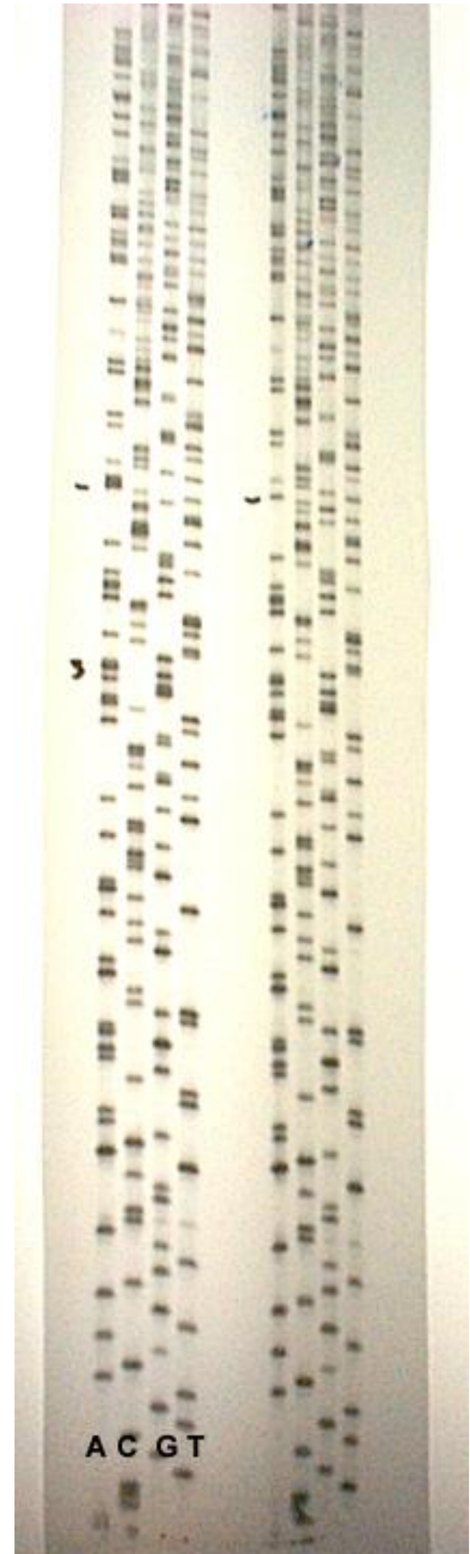
Another difference between older techniques and the current ones has to do with how you detect all the DNA molecules. In older methods, every DNA molecule was labeled with radioactivity. After electrophoresis, the gel would be placed against a piece of X-ray film for a period of time from hours to days (depending on the amount of radioactivity present). When the X-ray film was developed, each piece of DNA would show up as a band (dark area) on the film. A copy of one of these films can be seen on page 11.

Modern methods use fluorescent dyes instead of radioisotopes to label the DNA strands. The labeled DNA fragments are allowed to run off the bottom of the gel, where they are detected and the data fed directly into a computer. The resultant chromatogram has a peak for each nucleotide. To see samples of chromatograms, visit the following website:

http://cancer-seqbase.uchicago.edu/traces.html

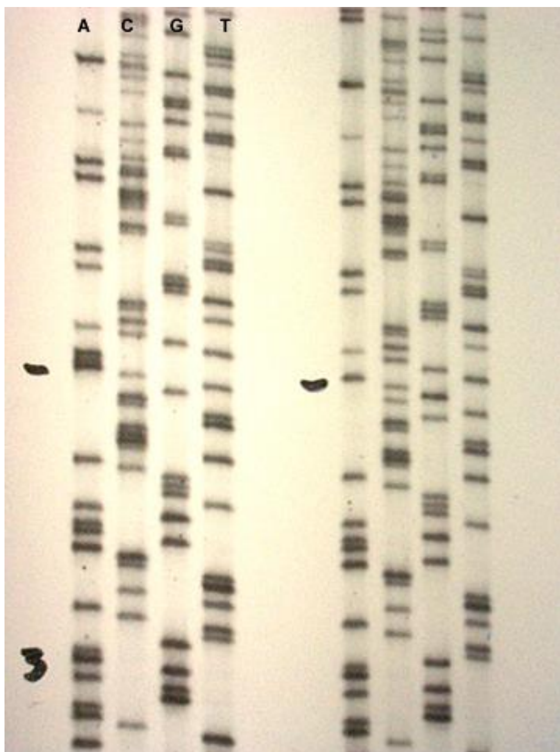
Sequences determined from the sample sequencing film (page 11) can be used for a BLAST search on the web; it should match a known DNA sequence that codes for protein. The website is here: nucleotide BLAST

DNA SEQUENCING FILMS



To the right is a scanned copy of a DNA sequencing film. The film shows DNA sequence from 2 samples of DNA. Each sample has 4 lanes on the film, one for each of the 4 bases, A, C, G, and T. The sequence can be determined by reading up the "ladder" from the bottom. For example, if you begin reading the sequence of the first DNA sample right above the letter "C" at the bottom of the film, the sequence reads: CTGTACGATG. (The lowest band, right above the letter, is a C. The next band up the gel is in the T lane, the one after than is in the G lane, etc.)

If you look closely at the two sequences, you'll see that they are identical except in one area (marked with a small dash). The second sample is the same as the first DNA sample except that the second sample has a mutation. This is a sequence from the laboratory of Dr. Dennis Dean at Virginia Tech, and the change in the DNA sequence, the mutation, was caused deliberately in the laboratory. This is a common method for studying a protein (the product of the DNA). The DNA for a protein is mutated, then the original protein (the "wild-type" protein) is compared with the mutant protein. Below is a close-up of the area of the sequence containing the mutation.



CONDUCTING ELECTROPHORESIS: BACKGROUND INFORMATION

When DNA is digested by restriction enzymes, the result is a DNA solution that contains DNA fragments of varying sizes. The number of fragments and the sizes of the fragments depend on the restriction enzyme used and the size of the original DNA molecule. In order to determine what the DNA fragment sizes are, it is necessary to: (1) separate the fragments by size; (2) have some way to visualize the DNA; and (3) have a standard to which the fragments can be compared. The first is accomplished by separating the DNA using agarose gel electrophoresis.

Electrophoresis is the movement of a charged molecule in an electrical field. A charged molecule will migrate toward the electrode of opposite charge. Since DNA molecules are negatively charged, they will migrate toward the anode (positive electrode).

If electrophoresis were done without a solid matrix (e.g., agarose), the rate of migration would be determined strictly by the charge:mass ratio of the molecules. Since DNA is a repeating polymer, DNA molecules of all sizes have the same charge:mass ratio. (If an electrical current were to be applied to DNA in a buffer solution alone, the DNA molecules would all migrate toward the anode at the same rate.) Thus, it is necessary to add a matrix such as agarose or acrylamide to act as a sieve and separate the DNA molecules based on their size.

The choice of matrix, agarose or acrylamide, is determined by the sizes of the molecules to be separated. Acrylamide is used primarily to separate proteins and small DNA molecules (under 1000 base pairs). Agarose is the matrix used to separate most DNA molecules.

Agarose is a polysaccharide (from algae) that can be dissolved in hot water. As the agarose solution cools, it solidifies to form a matrix of gelatin-like consistency. The matrix contains pores through which the DNA molecules must pass. The size of the pores, and hence the sizes of the DNA molecules that can be separated on the gel, is determined by the concentration of the agarose solution. For example, large DNA molecules (>10,000 base pairs) can best be separated on a 0.3% agarose gels (e.g., larger pores), whereas small DNA molecules (100-3000 base pairs) would separate with better resolution on a 2.0% agarose gel (e.g., smaller pores). In the experiments in this kit, 0.9% agarose gels will be used to separate the DNA molecules.

As these 0.9% gels are prepared, a comb is placed in the gel at the end closest to the cathode (negative electrode). After the agarose solution has solidified, the comb can be removed, leaving small holes or *wells* in the gel into which the samples will be loaded. The DNA samples are mixed with a loading buffer that contains glycerol and a tracking dye. The glycerol adds density to the samples, assuring that they will stay in the wells when loaded. The tracking dye usually contains a dye like bromphenol blue, a small molecule that migrates through the gel at a position approximately equivalent to a DNA fragment of 300 base pairs, or Orange G, which migrates through the gel at a position approximately equivalent to a DNA fragment of 50 base pairs. The dyes serve two functions. They makes it easier to see the samples while the wells are being loaded and, since the dye can be seen as it migrates through the gel, it can be used to estimate how far the DNA has migrated in the gel.

When it is time to load and run the gel, the gel is covered in buffer, the comb carefully removed, and the samples loaded into the wells. A standard solution consisting of DNA fragments of known sizes is loaded into an adjacent well. The lid is placed on the gel box, the gel box is connected to a power

supply, and an electrical current is passed through the gel. The DNA molecules immediately begin to migrate toward the anode, with smaller molecules migrating more rapidly than larger DNA molecules (Figure 1).

It is necessary to have some method for visualizing the DNA in the agarose gel. In the research lab, fluorescent stains are frequently used. Two commonly used stains are ethidium bromide and GelRed. Either stain can be added to the gel and/or buffer solution or the gel can be post-stained in a dye solution. Either way, the dye is inserted between the stacked bases of DNA and glows bright orange or red when the gel is exposed to UV light. UV transilluminators, used to view the gels, are expensive and must be used carefully to avoid UV exposure, so they are uncommon in the high school classroom. In addition, ethidium bromide is a mutagen and hazardous to handle. GelRed is safer, as it does not penetrate the skin.

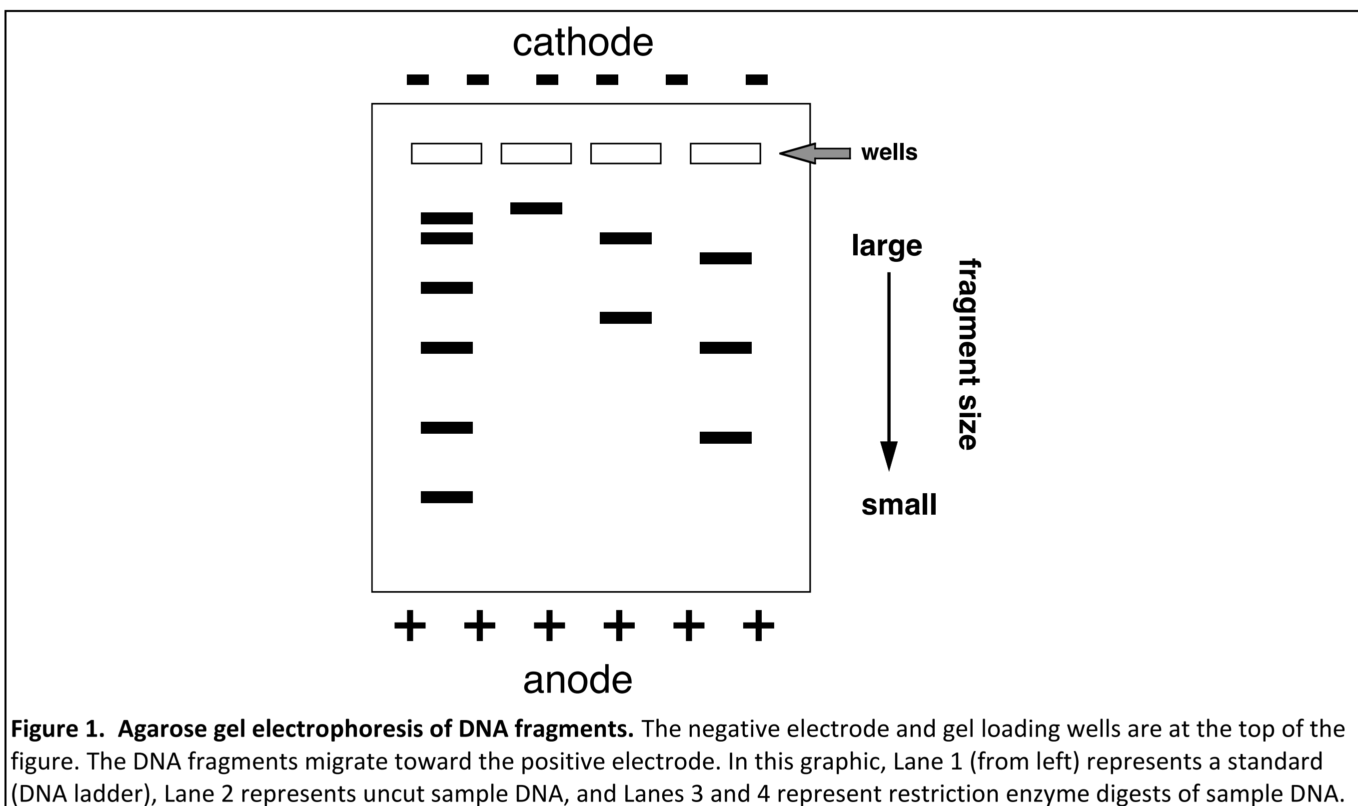


Figure 1. Agarose gel electrophoresis of DNA fragments. The negative electrode and gel loading wells are at the top of the figure. The DNA fragments migrate toward the positive electrode. In this graphic, Lane 1 (from left) represents a standard (DNA ladder), Lane 2 represents uncut sample DNA, and Lanes 3 and 4 represent restriction enzyme digests of sample DNA.

In the following procedures, methylene blue is used as a post-stain for DNA. Although methylene blue is not as sensitive as ethidium bromide, it is less hazardous. The DNA bands appear blue on a clear background and the migration of the fragments can be measured. Using the migration distances of the DNA fragments in the standard, a standard curve can be generated and the sizes of the DNA fragments from the experimental restriction digests can be calculated (see below for details).

STUDENT PRE-LAB ACTIVITY: WHAT IS DNA? DNA EXTRACTION FROM STRAWBERRY

Teacher Guide

Introduction

DNA is present in the cells of all living organisms. This procedure is designed to extract DNA from a strawberry in sufficient quantity to be seen and spooled. This activity is ideal for students to work in pairs, but each student will have a tube of DNA at the end.

Some questions to get you thinking about today's lab:

1. One way to purify a molecule is to get rid of everything but that molecule. If we want to isolate DNA from a strawberry, what do we have to get rid of?

   All parts of the cell besides the DNA, e.g., cell wall (a strawberry is a plant, after all), cell membrane, mitochondria, Golgi apparatus, endoplasmic reticulum, vacuoles, lysosomes, nuclear membrane, etc.

2. What materials would you use to do that?

   Something to mush the cells (blender or your hands), something to destroy membranes (soap dissolves them), something to get rid of proteins and carbohydrates (salt causes them to precipitate), something to separate insoluble cell stuff from soluble DNA, and something to help get the DNA (alcohol precipitates it).

3. What can we do with the DNA once we've purified it?

   Use it in DNA fingerprinting (solve a crime, see a genetic defect), put it into another organism to give it specific traits (this is called transformation or genetic engineering), other?

Materials:

- Ziploc bags (1 per pair)
- strainer or funnel (1 per pair)
- cheesecloth (cut to cover the funnel)
- extraction solution (20 ml in tube per pair)
- strawberries
- small test tubes (2 per pair)
- cold 95% ethanol (about 5-10 ml per student) Note: Isopropanol (rubbing alcohol) will also work, just not as well as ethanol

Extraction solution recipe:

For one liter of the extraction solution, mix 100 ml of shampoo (do not use shampoos with conditioner or baby shampoo) and 15 g of table salt (iodized or non-iodized — both will work). Add water to make a final volume of 1 liter. Dissolve the salt by stirring slowly to avoid foaming. Measure 20 ml of solution for each pair of students.

Protocol

1. Put a strawberry in a Ziploc bag.

2. Add 20 ml of extraction solution and close the bag without much extra air. Mush the strawberry thoroughly but carefully so the bag doesn't break, for about 5 minutes. What does mushing the strawberry do?

   Breaks the cell wall.

3. What do you think the extraction solution is? What does it do to the strawberry?

   Soap will cause the solution to bubble so students should be able to guess what's in here. The soap destroys the cell and nuclear membranes, allowing the DNA to get out. There is also salt in the extraction solution, which causes the proteins and carbohydrates to precipitate, while the DNA remains in solution.

4. Filter the mixture through the cheesecloth. (All of the groups can combine their mixtures at this point to filter together, or each can filter separately if there are enough funnels and cheesecloth available.) What is being filtered out? What is going through the filter?

   Students can usually see the seeds being filtered out. Most of the cell parts and the precipitated protein and carbohydrate are also being filtered out at this point.

5. Each student should have at least 5 ml of strawberry solution in a test tube.

6. **Being careful** not to shake the tubes and add approximately 5 ml of cold 95% ethanol to each tube. What do you think the ethanol does? Why do we want it cold?

   The goal here is to precipitate (or solidify) the DNA. The colder something is, the more likely it will precipitate or solidify. Cooling the alcohol just increases the amount of DNA that precipitates.

7. Take a look at your tube. What do you see in the top portion of the liquid?

   You can actually pick up the DNA at this point, using a toothpick, wood pencil, or glass stirring rod.

8. What can DNA be used for once it is isolated?

   See the Real-World Applications section of the Appendix (page 35) for ideas.

STUDENT PRE-LAB ACTIVITY: WHAT IS DNA?
DNA EXTRACTION FROM STRAWBERRY

Introduction

DNA is present in the cells of all living organisms. This procedure is designed to extract DNA from a strawberry in sufficient quantity to be seen and spooled.

Some questions to get you thinking about today's lab:

One way to purify a molecule is to get rid of everything but that molecule. If we want to isolate DNA from strawberry, what do we have to get rid of?

What materials would you use to do that?

What can we do with the DNA once we've purified it?

Materials:

Ziploc bag
strawberries
strainer or funnel
cold 95% ethanol
cheesecloth
small test tubes
extraction solution

Protocol

1. Put a strawberry in a Ziploc bag.

2. Add 20 ml of extraction solution and close the bag without much extra air. Mush the strawberry thoroughly but carefully so the bag doesn't break, for about 5 minutes. What does mushing the strawberry do?

3. What do you think the extraction solution is? What does it do to the strawberry?

4. Filter the mixture through the cheesecloth. What is being filtered out? What is going through the filter?

5. Each student should have at least 5 ml of strawberry solution in a test tube. **Being careful** not to shake the tubes and add approximately 5 ml of cold 95% ethanol to each tube. What do you think the ethanol does? Why do we want it cold?

6. Take a look at your tube. What do you see in the top portion of the liquid?

7. What can DNA be used for once it is isolated?

STUDENT PRE-LAB ACTIVITY: PIPETTING PRACTICE AND PRACTICE GEL LOADING

Materials:

- fixed volume pipettors
- microcentrifuge tube racks
- microcentrifuge tubes
- yellow pipette tips
- dye solution
- water
- practice loading gels
- practice loading dye
- 12 µl aliquots of practice dye in microcentrifuge tubes.

Preparation required:

- Any liquid can be used for pipetting practice, but it can make it easier initially to use a colored solution. Water with food coloring will work, or dilute some of the 50x methylene blue for the students to use. Give each student 0.5–1.0 ml of dye in a microcentrifuge tube, plus a tube containing water and an empty tube or two.
- For practice gel loading, use the practice loading dye provided.
- When the students are loading their real DNA samples on the agarose gels, they will be pipetting and loading a 10 µl aliquot, i.e., not removing 10 µl from a much larger volume. It would be a good idea if they tried this first during the practice pipetting.
- For the most realistic practice, place the practice loading gels into a container so that they can be submerged in water. The water should be deep enough so that no "dimpling" is seen over the wells.

Procedure:

1. **Practice pipetting:** Have the students practice pipetting dye solution both into empty microcentrifuge tubes and into microcentrifuge tubes containing water. When pipetting into an empty tube, touch the end of the pipette tip on the side wall of the tube and expel the sample by depressing the plunger gently. To pipette a sample into another solution, place the pipette tip into the solution and then dispense.
2. **Practice gel loading:** Have the students practice loading the "wells" of the practice loading gels with practice loading dye. When they do the real thing, they will be loading 10 µl (precut DNA), so that would be a good volume with which to practice.

Points to stress:

- The dye is denser than water (or gel buffer). If the sample is expelled at the top of, or even over, the well, it will drop into the well. There is no need to plunge the pipette tip into the bottom of the well. **Piercing the bottom of the well when the real gels are loaded will result in lost sample.**

Clean up:

- Rinse the practice loading gels with water to remove the dye.
- Shake the gels to remove excess water.
- If you have time and space, leave the gels sitting out so that they air-dry before repacking them.

ANALYZING DNA WITH ELECTROPHORESIS

Suggestion: Make the lesson more interesting for your students by using one of the mystery scenarios found on pages 44–58. Let them answer the question of who has been stealing the cat food from Fluffy's bowl, who killed the delivery man, or do Susan and Tom carry defective alleles of the cystic fibrosis gene?

Materials:

- pHokie plasmid DNA. The DNA has been digested with the restriction enzymes EcoRI, PstI, and with both enzymes. The samples already contain Orange G loading dye.
- 1 kb Plus DNA ladder
- 10 µl fixed volume pipettors
- yellow pipette tips

Preparation required:

Aliquoting reagents: Here are the amounts to be aliquoted for each student group. If the reagents are aliquoted into colored centrifuge tubes, then the tubes need not be labeled individually.

- pHokie digested with EcoRI — 1 tube containing 10 µl
- pHokie digested with PstI — 1 tube containing 10 µl
- pHokie digested with EcoRI and PstI — 1 tube containing 10 µl
- 1 kb Plus DNA ladder (optional) — 1 tube containing 10 µl

If using a mystery scenario, you will also need:

- mixture of pHokie double digest *& PstI digest* — 1 tube containing 10 µl

Procedure:

1. Prepare an agarose gel for electrophoresis.

2. Load the contents of each tube into a separate well, using a new pipette tip for each. (Optional) Load 10 µl of the 1 kb DNA ladder in an adjacent well. Record the order in which the samples are loaded.

3. Place the lid on the gel box. The black lead must be at the same end of the gel as the samples. Connect the leads to the power supply, red to red and black to black. Turn on the power supply and adjust the voltage as instructed by your teacher. Check the electrodes to be sure that bubbles are rising from the wires. If no bubbles can be seen, double-check the connections.

4. Run the gel for until the dye front nears the bottom of the gel. Stain the gel as described below.

STAINING AN AGAROSE GEL WITH METHYLENE BLUE

Preparation required:

Diluting methylene blue stain: The stock methylene blue solution is 50x, so dilute 1:50 to get the 1x working concentration use. Each gel requires ~50 ml for staining, so add 1 ml of 50x stain to 49 ml water. **Note**: Methylene blue stain is reusable.

Procedure:

1. After the gel has electrophoresed for the desired amount of time, turn off the power supply and disconnect the leads.
2. Remove the gel box lid and remove the gel tray from the gel box. **Note**: Be careful that the gel does not slide off the tray prematurely.
3. Slide the gel into a plastic box for staining and destaining. Stain the gel in 1x methylene blue solution by soaking the gel in stain for 15–20 minutes.
4. To destain the gel, pour off the methylene blue and rinse the gel several times with water. Add fresh water to the box. **Important note:** Add just enough water to cover the gel; **do not use too much water!** More is *not* better in this case.
5. Let the gel soak in the water for several minutes, periodically rocking the box gently. Change the water, and repeat. Continue until the gel is adequately destained. More changes of fresh water will improve destaining, as will agitating the gel during destaining.
6. Bands should be visible after 15–30 min of destaining, although longer destaining will improve signal to noise ratio, i.e., reduce the background staining. (See note below.)
7. View the staining on a light box. Either cover the box with plastic wrap or place the gel in a plastic bag to prevent staining the light box surface. Analyze the data as described below.

Notes:

- The most important consideration in destaining the gel is to use minimal amounts of water, i.e., no deeper than *just* covering the gel. If you flood the gel with water, especially for longer periods like overnight, you can wash the stain right out of the gel.
- The best way to destain is to do multiple changes of small volumes of fresh water over a longer time period, periodically rocking the box. If you can't get it done during the class period, then perhaps send the gels home with the students, telling them to continue the destaining during the evening. If you leave the gels sitting overnight or longer, just leave them in a minimal amount of water sealed in the boxes.
- The methylene blue stain can be used repeatedly. The 1x working concentration is 0.025% methylene blue, hence the 50x stock is 1.25% (= 1.25 g per 100 ml water).

ANALYZING DATA FROM AN AGAROSE GEL

Analysis procedures can be as simple as determining the guilty party (or cat) if you followed a crime scene scenario. Which of the “suspect’s” DNA matches that found at the “crime scene.”

If your students have run the optional DNA ladder on their gels, you may go further with the analysis and have your students measure the migration of the DNA bands, plot a standard curve, and determine the sizes of the DNA fragments from the enzyme digests. A final step, also optional, is to use the data to create a simple map of the plasmid.

Procedure (optional): Constructing a standard curve and determining the sizes of the DNA fragments from the enzyme digests

1. Cover the light box with plastic wrap or place the gel in a plastic sandwich bag. Place the gel on the light box.
2. First look at the lane containing the 1 kb Plus DNA ladder. Compare it to a picture of the bands with their known sizes. To figure out which band is which, look at the figure, and find the two bands that are 1650 and 2000 base pairs. Notice that these two bands are separated from the other bands, and rather easy to find.
3. Find these two bands on the stained gel and measure their migration from their point of origin in the gel, i.e., from the well. Measure from the bottom the well to the foremost edge of the stained band. Be certain to measure each from the same point, e.g., from the bottom of the well each time, not the bottom one time and the top of the well the next. Record the base pair size of the band and its migration distance.
4. Working up (toward larger DNA fragments) and down (toward smaller DNA fragments) from the 1650 and 2000 bp bands, measure and record the migration of the other bands in the DNA ladder.

   The large bands will be too close together to be measured accurately, and the smaller bands may have migrated off the bottom of the gel. Remember, if you have run the gel until the dye has reached the bottom of the gel, then anything smaller than dye (50 bp for Orange G) will have run off the bottom of the gel.
5. Measure the migration of the bands in the experimental lanes and record the migration distances.
6. Create a standard curve using the data from the 1 kb DNA ladder. Graph the migration distance of the DNA fragments (x-axis) against the size of the DNA fragments (y-axis) on semi-log graph paper. Connect the points to form a line.
7. To determine the size of an enzyme-digested DNA fragment, find where the migration distance of the DNA fragment intersects the standard curve. Draw a line from this point to the y-axis. Where this line meets the y-axis is the size of the fragment.

Procedure: constructing a map of the plasmid

1. You can use the data (the sizes of the fragments from the three digests) to construct a map of the plasmid. Look at the results from the EcoRI digest. Now draw a circle to represent the plasmid and place arrows on the circle to show where the enzyme must have cut. Put an "E" next to each arrow. (It doesn't matter where you put the first arrow on the circle. You don't have any information about that; all you can tell is where the enzyme cuts are in relation to each other.)
2. Now look at the results of the PstI digest. How many bands are there? Can you explain this result? Since pHokie is a plasmid, a circular piece of DNA, what will happen if an enzyme has only a single recognition site on the plasmid?
3. Finally, look at the double digest. Place an arrow on your plasmid circle to mark where PstI cut the plasmid in relation to the EcoRI arrows. Put an "P" next to the PstI arrow.
4. What you have constructed is called a DNA restriction map. This is a very simple map of a small plasmid. When maps are constructed in a research laboratory, they will frequently feature 10 or more restriction enzymes, each of which may have multiple recognition sites.

Notes:

- Gel analysis can be made easier if you have a digital camera, cell phone camera, or scanner available. Photograph or scan the students' gels and give them a printout on which to make their measurements. There are several advantages to this. First, measurements made on a print will be more accurate than those made on the gel itself, especially if you enlarge the size of the gel in the print. Second, each student could then work independently on the analysis, and then the individuals could compare their results. This would be a good way to introduce the concept of experimental error and the need for replication.
- When the standard curve is constructed using the data from the 1 kb DNA ladder, you will find that the data are more linear for the smaller fragments than at the upper end of the curve. As a result, molecular weight calculations for the experiments (the enzyme-digested DNA) will be closer to expected values for smaller fragments than for larger fragments.

Resources:

- A picture of the bands of the 1 kb Plus DNA Ladder and their known sizes in base pairs (bp) can be found on page 32. A sheet of semi-log graph paper is on page 66.
- The known sizes of the DNA fragments generated by the restriction digest of the pHokie plasmid are on page 31. A map of the plasmid is also pictured.

CLEANING UP

Cleaning the gel boxes and trays:

- The gel boxes and trays (and lids also, if they are dirty) should be rinsed in water to remove the buffer residue. Do **not** scrub the gel box; rinse it only. The electrode wires are easy to break and expensive to replace, as they are platinum.

- Place the items on paper towels or something similar to air dry. Do not attempt to dry the gel boxes, again because of the platinum electrodes.

- Save any unused yellow tips and tubes to send back with the kit. Do not mix used and unused items.

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USE OF THE FISHERBRAND MICROPIPETTORS

IMPORTANT NOTE: Please be very careful when using these pipettors. They are provided for the instructor to use to aliquot the materials needed for the experiments.

The FisherBrand pipettor measures from 5-40 µl. The volume is adjusted by turning the plunger knob. You can feel/hear clicking as the knob is turned. Turn the knob clockwise to decrease the volume setting and counterclockwise to increase the volume setting. The volume setting is displayed digitally on the handle of the pipettor.

The volume readout is in microliters.

- The 5–40 µl pipettor has an operating range of 5–40 µl and the scale division (each click) is 0.5 µl. For example, to pipette 15 µl, turn the knob until the digital display reads 15.0. Do **not** turn this pipettor above 40.0 or below 5.0.

NOTE: The biggest problem we've had with the pipettors is their being turned out of their range! ***Never*** force the adjustment knob and never turn the knob beyond the range of the micropipettor! If the knob does not turn easily, assume that you are making a mistake — check the setting!

STEPS FOR USING AN FISHERBRAND MICROPIPETTOR:

1. Set the pipettor to the desired volume as described above.
2. Place a tip on the pipettor.
3. The plungers on FisherBrand pipettors have 2 stops. The first is used to draw liquid into the tip and the first and second stops are used to dispense liquids.
4. To draw liquid into the pipette tip, press the plunger down to the first stop and place the pipette tip in the liquid to be pipetted. Slowly release the plunger, drawing liquid into the tip. Do not allow the plunger to pop up. This can draw liquid into the pipettor itself. **NOTE: Be sure that you are only depressing the pipettor plunger to the FIRST stop.** If you are going to the second stop, you are pipetting far more volume than you intend to pipette.
5. Withdraw the tip from the sample solution and dispense the sample from the pipettor. To dispense into an empty vessel, touch the end of the pipette tip on the side wall of the vessel and depress the plunger to the first stop. Wait ~1 second and then continue to depress the plunger to the second stop. If you are pipetting a sample into another solution, place the pipette tip into the solution and then dispense.
6. Without removing pressure from the plunger, withdraw the tip from the tube or solution, and then allow the plunger to return to the up position. If you release the pressure on the plunger while the tip is in the solution, you will pull solution into the tip again.
7. Eject the used tip by pressing down on the tip ejector located on the side of the pipettor handle.

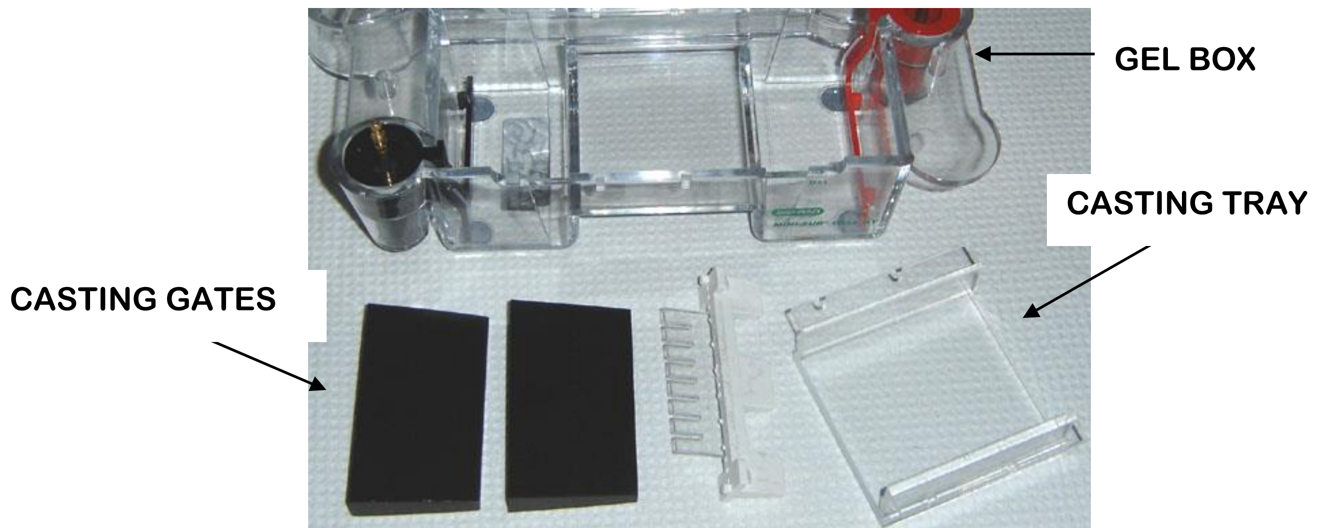
STEPS FOR USING FIXED VOLUME PIPETTORS:

1. Place a yellow tip on the pipettor by twisting the tip on while applying pressure. Be sure the tip is on the pipettor tightly.
2. To draw liquid into the pipette tip, press the plunger down until it stops and place the pipette tip in the liquid to be pipetted. Release the plunger, drawing liquid into the tip.
3. Withdraw the tip from the sample solution and dispense the sample from the pipettor. To dispense into an empty vessel, touch the end of the pipette tip on the side wall of the vessel and depress the plunger down until it stops. If you are pipetting a sample into another solution, place the pipette tip into the solution and then dispense.
4. Without removing pressure from the plunger, withdraw the tip from the tube or solution, and then allow the plunger to return to the up position. If you release the pressure on the plunger while the tip is in the solution, you will pull solution into the tip again.

Note on aliquoting reagents: The reagents in this kit are expensive. We do add a "cushion" to the volumes we send (an extra 20% above the volume needed), but not enough to support poor pipetting. There are 3 ways to be sure you have enough material:

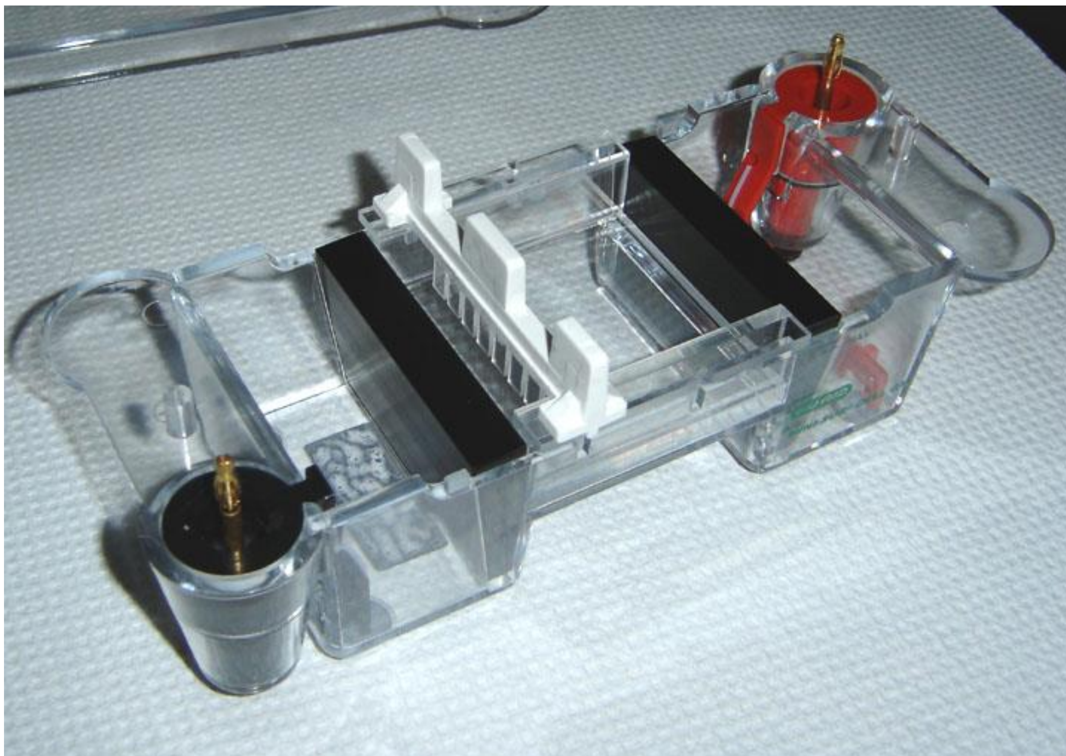
1. Before you aliquot any liquid reagents, bring the contents to the bottom of the tube by spinning the tube briefly in the microcentrifuge.
2. Be certain that you understand the operation of the Fisher Micropipettor (see page 24). If you depress the plunger to the wrong position, you will pipette much more volume than intended and not have enough for the experiment.
3. While pipetting, wipe liquid on the outside of the pipette tip on the inner wall of the microcentrifuge tube. You may need to spin the contents to the bottom of the tube again during aliquoting.

CONDUCTING ELECTROPHORESIS: SETTING UP THE GEL BOX



Form the casting chamber by:

- Placing the casting tray on the gel box base with the comb slots at the end with the black electrode (cathode).
- Slide the casting gates into the slots on either side of the gel tray.
- Place the comb in the slot closest to the black electrode.



CONDUCTING ELECTROPHORESIS: POURING GELS

Options:

- Instructor prepares agarose and pours all the gels.
- Instructor prepares the agarose and students pour the gels.
- Students prepare agarose and pour gels.

Diluting the buffer to *prepare* the agarose gels:

With the change to TAE buffer, this is slightly more complicated than it was with the SB buffer. The TAE buffer is provided as a 50x stock. You will use a 1x concentration to prepare the gels and a 0.25x concentration to run them. (Note: a gel prepared with 0.25x rather than 1x TAE will not run correctly; if you use 1x TAE as running buffer, the gel will run, but you must lower the voltage to 100 volts or the gel will melt.)

Dilute 50x TAE to 1x concentration. Dilute 1:50 in water (distilled, if available) for the 1x concentration. You will need 30 ml of 1x TAE to prepare each 30 ml agarose gel.

To prepare 1x TAE:

For	1 gel	4 gels**	8 gels** (1 class)	16 gels** (2 classes)	24 gels** (3 classes)	32 gels** (4 classes)	etc.
50x TAE	600 µl	3 ml	6 ml	12 ml	18 ml	24 ml	
water	29.4 ml	147 ml	294 ml	588 ml	882 ml	1,176 ml	

** includes a cushion — more volume than you will need to prepare the gels.

Diluting the buffer to *run* the agarose gels:

You will use a 0.25x concentration to run the gels.

Dilute 50x TAE to 0.25x concentration. Dilute 1:200 in water (distilled, if available) for the 0.25x concentration. You will need 300 ml of 0.25x TAE to run each gel.

To prepare 0.25x TAE:

For	1 gel	4 gels**	8 gels** (1 class)	16 gels** (2 classes)	24 gels** (3 classes)	32 gels** (4 classes)	etc.
50x TAE	1.5 ml	8 ml	16 ml	32 ml	48 ml	64 ml	
water	294 ml	1.6 liters	3.2 liters	6.4 liters	9.6 liters	12.8 liters	

** includes a cushion — more volume than you will need to run the gels.

Preparing a 0.9% agarose gel:

1) Prepare the gel box as described above (page 26).
2) Add weighed agarose to 1x TAE buffer in a glass flask or bottle. Do not swirl the solution, as the undissolved agarose will stick to the sides of the flask.

gel box type	1x TAE (ml)	agarose (g)
Bio-Rad	30 ml	0.27

   If you are pouring more than one gel, just multiply by the number of gels and use a larger container.
3) Heat the solution in a microwave oven to dissolve the agarose.
   a) Do not seal the container, and watch carefully that the solution does not boil over.
   b) When the hot agarose solution is removed from the microwave, it may be superheated. Carefully remove the flask from the microwave using the thermal grippers and, pointing the flask away from yourself and others, swirl gently. The superheated solution may bubble briefly at this point. (**Be careful,** as it may boil over, out of the flask!)
   c) As the agarose dissolves, the solution will become clear. Swirl the flask to be certain that all the agarose is dissolved. If it is not, you will see little clear flecks floating in the solution. Hold the flask up to the light and examine the solution carefully to be sure that the agarose is all dissolved.

   Note: if a microwave is not available, the solution can be heated in a boiling water bath or on a hot plate.

   While heating the solution, some of the volume will be lost to evaporation. More will be lost when the uncovered solution is microwaved; frequently, 20-30% of the volume is lost. One solution is to weigh the flask before heating and then, after heating, add water to bring the solution back to the original weight. Another solution is to mark the volume level on the flask prior to heating, and then add water to bring the solution back to that level. After the water is added, swirl to mix the solution.
4) Cool the agarose solution to 55-60˚C before pouring the solution into the prepared gel casting tray. The cooling can be done either by placing the flask in a 60˚C water bath, or by leaving the flask at room temperature until the solution has cooled.
5) Place the casting tray on a level surface and pour the cooled agarose solution gently into the tray, avoiding bubbles if possible. Insert the comb, if not already in place. Do not move the tray while the gel is solidifying. When the gel is ready, it will be somewhat translucent and firm to the touch. This takes from 15–30 min.

Notes:

1. If the instructor is preparing the agarose solution, but wants the students to pour the gels, the agarose solution can be left at 60˚C in the water bath for extended periods. (I've left it overnight with no problem.) Be sure you cover the flask well. Then, give the students aliquots to pour their gels.
2. The gels can be poured one day and used on another day. After the gels are poured and have solidified, place the gel tray in a container that can be sealed, e.g., a large Rubbermaid container or large sealable plastic bags. Place wet paper towels in the bottom of the container. They will keep the moisture level high in the container and prevent the gels from drying out.

CONDUCTING ELECTROPHORESIS: RUNNING THE GEL

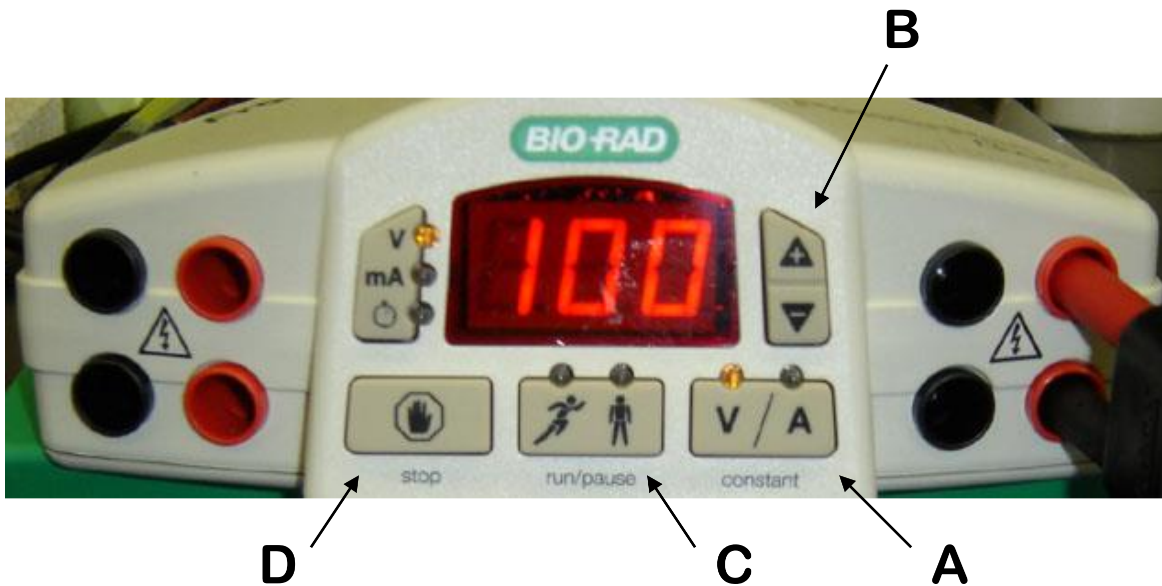
1. When the agarose is set, gently remove the comb from the gel.
2. Remove the metal casting gates. (**Don't forget to do this!!)**
3. Pour the running buffer (0.25x TAE buffer) into the buffer chamber until the buffer covers the top of the gel. If you see any "dimpling" in the buffer over the wells, add a little more buffer.
4. Load the gel. Each well will hold 20–25 µl of sample.
5. Place the lid on the gel box.
6. Connect the leads to the power supply, red to red and black to black.
7. Start the run (power supply directions are below). Gels being run in 0.25x TAE can be run at up to 200 volts, which gives a run time of under 20 minutes.

   You do not **have** to run the gels at 200 volts. If you want to run them at the old speed of 100 volts, or anything between 100 and 200 volts, it will work just fine.

8. Check the electrodes to be sure that bubbles are rising from the wires. If no bubbles can be seen, double-check the connections.
9. Run the gel until the dye front nears the bottom of the gel. Stain the gel as described on page 19.

OPERATION OF POWER SUPPLIES

Bio-Rad PowerPac Basic power supplies have digital readouts and are fully adjustable. Please double-check the settings before starting electrophoresis!



To use:

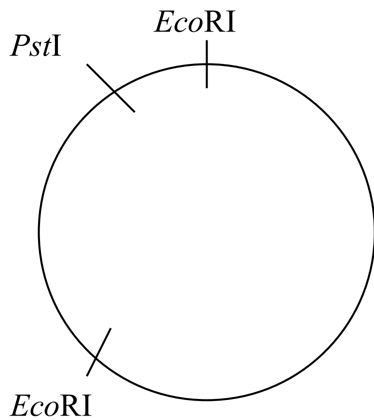
1. Plug in gel box leads, matching red to red and black to black.
2. Turn power on using the switch on the right side of the unit. Note: If the display flashes E-7, turn the power off and back on, and the error message should clear.
3. The V light (A) should be on (the unit defaults to constant voltage). If it is not, push the Constant button until the V light comes on.
4. Enter the desired voltage by scrolling up with the arrow keys (B) on the right of the digital readout.
5. Start the power by pushing the Run/Pause button (C).
6. When the run is finished, push the Stop button (D).

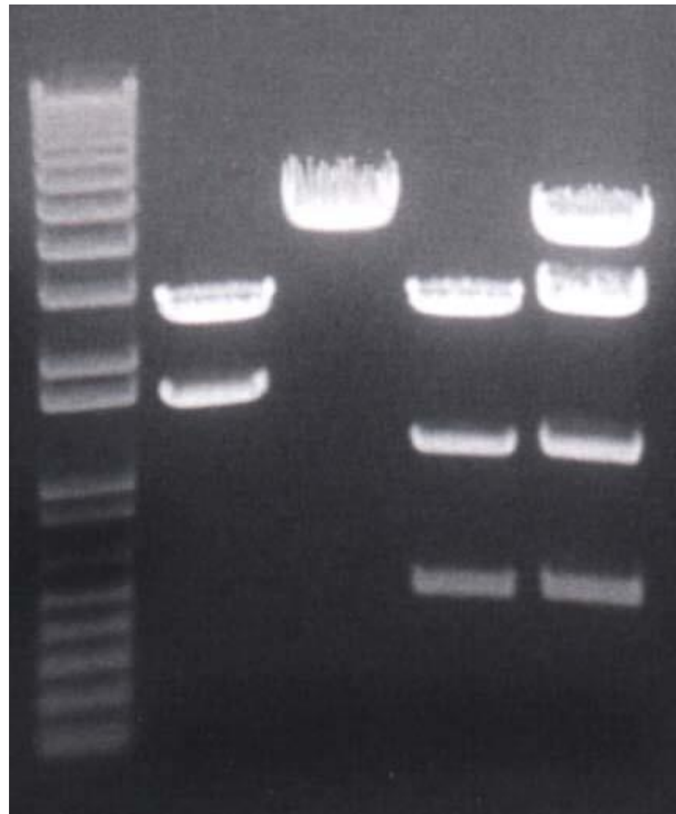
SIZE OF DNA FRAGMENTS GENERATED BY RESTRICTION DIGESTS OF pHOKIE PLASMID DNA

fragment	size (in basepairs)
intact pHokie	4200
EcoRI digest	2700 1500
PstI digest	4200
EcoRI & PstI double digest	2700 1100 400
Crime scene sample (mixture of PstI digest and EcoRI & PstI double digest)	4200 2700 1100 400

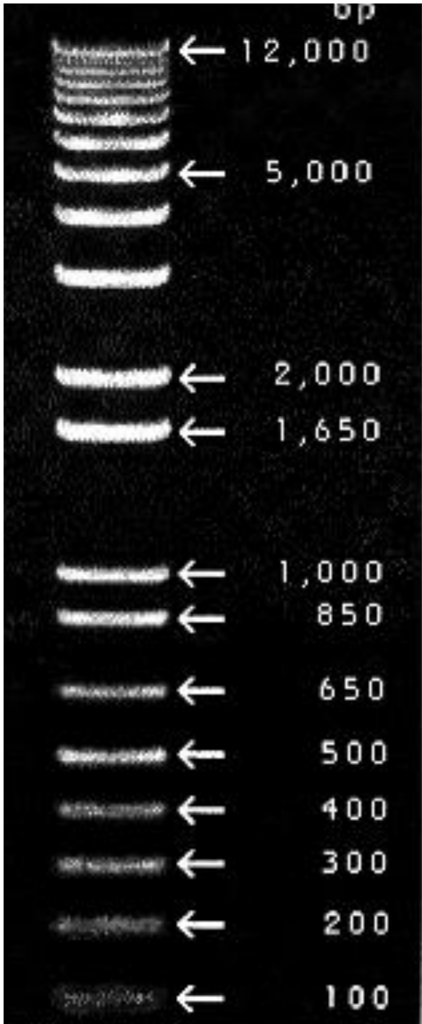
Sample gel and plasmid map

Lane 1 DNA ladder
Lane 2 EcoRI digest
Lane 3 PstI digest
Lane 4 EcoRI & PstI double digest
Lane 5 Crime scene sample





1 kb PLUS DNA LADDER (optional)



CONCEPTUAL REVIEW MATERIALS

Three different icons have been placed throughout the manual to note points at which students can be asked Thinking Questions (signified by a question mark); Real-World Applications (signified by a globe); and Ethical, Legal, and Social Issues (ELSI, signified by a balance).

Thinking Questions

These questions are designed to prompt deeper thinking about the concepts covered.

1) Restriction enzymes are primarily isolated from bacteria. Why would bacteria have enzymes that cut DNA? How do the bacteria avoid cutting up their own DNA with the restriction enzymes they make?

It is commonly thought that bacteria use restriction enzymes as a primitive 'immune system', by using these DNA-destroying enzymes to cut up viral DNA. Bacterial DNA does not have methyl groups attached to it, whereas viral and eukaryotic DNA does. Many restriction enzymes only cut methylated DNA, and thus won't harm bacterial DNA.

2) Which is larger - a strand of DNA or a single protein?

Obviously, it depends on the strand of DNA and the protein in question. Yet, a DNA strand typically contains more than one gene, and, therefore, codes for multiple proteins. Thus, DNA is generally larger than most proteins.

3) Why is the DNA loaded at the negative (black) electrode of the gel?

Deoxyribonucleic acid is, obviously, an acid, which means that it is a proton (H^+) donor. If DNA gives away a proton, which is positively charged, the remaining DNA molecule will be negatively charged. If the DNA is loaded at the negative electrode in the electrophoresis set-up, it will migrate through the gel toward the positive electrode.

4) Why do bubbles form on the electrodes in the electrophoresis set-up? What are the bubbles?

Electrophoresis involves a redox reaction - H^+ from the water is being reduced to form hydrogen gas (H_2) and O^{2-} from the water is being oxidized to form oxygen gas (O_2). Because H^+ is positively charged, it will migrate to and be reduced at the negative electrode (black). The O^{2-} will migrate and be oxidized at the positive electrode (red).

If the students look closely, they will be able to observe that there are more bubbles at the black electrode than at the red because there are 2 hydrogen atoms per oxygen atom in a water molecule, so there should be twice as much hydrogen gas than oxygen gas formed in this reaction.

Real-World Applications

The following are scenarios in which restriction enzyme analysis is used in real-life.

Restriction fragment length polymorphisms (RFLPs) can be used to identify DNA differences in individuals (humans, animals, trees, etc.). DNA is isolated from the individuals of interest, their DNA is cut and examined for any places where a restriction enzyme cuts the DNA of one individual but not another. This process is more commonly known as DNA Fingerprinting, and is done only rarely now because other approaches yield more accurate results with smaller tissue samples (e.g., a single cell).

Examples:

• Matching DNA found at a crime scene to DNA from a suspect

In this scenario, the DNA from the suspect and the crime scene must match exactly. Currently, forensic scientists examine 11-13 different sites in the human genome to identify a match. By examining multiple sites, the probability that there will be a match just by chance is virtually eliminated.

• Matching DNA from a child to DNA from a parent

In this scenario, half of the child's DNA will match its mother's DNA, and half will match its father's DNA. Typically, DNA is collected from the mother, the father, and the child and analyzed simultaneously to attribute every DNA band from the child either to the mother or the father.

This scenario is difficult to stage in a classroom because, unlike the crime scene sample that contains DNA from both the suspect and the victim, the child's sample would contain only a fraction of the DNA from either the mother or the father. Thus, simply mixing two samples does not provide a fair model for paternity testing.

Paternity testing is used not only in humans, but also in animals and even plants to determine an offspring's parentage or the genetic relationships between individuals in a population.

• Identifying human remains after a war or accident

In this scenario, DNA is isolated from the remains and attempts are made to match it to previously collected samples (e.g., blood samples taken before a soldier went to war) or to family members (similar to a paternity test).

Ethical, Legal, and Social Issues (ELSI)

Biotechnology, perhaps more than most fields in biology, is fraught with ethical, legal, and social implications. Although each district, school, and teacher must make decisions regarding how to handle ELSI education in their classes, biotechnology activities provide an ideal opportunity to address these issues within science classes.

Several online resources for ELSI education:

• Genetic Science Learning Center, University of Utah:
    http://learn.genetics.utah.edu/
    http://teach.genetics.utah.edu/

These websites feature genetics education materials, from information about basic genetics to case studies in genetics (e.g., stem cell use, biowarfare, etc.).

• High School Human Genome Project (HSHGP), University of Washington
    http://hshgp.genome.washington.edu/teacher_resources/modules.htm

This website is home to the ethics module about genetic testing developed by HSHGP personnel, which is freely available in PDF format.

• Dolan DNA Learning Center (DNALC), Cold Spring Harbor Laboratory
    http://www.dnalc.org/
    http://www.eugenicsarchive.org/eugenics/

The DNALC is home to a wealth of online resources, including a eugenics case study that can serve as the basis for discussion about the potential risks of genetic testing.

If you have educational materials regarding the ethical, legal, or social implications of biotechnology that you would be willing to share, please let us know. We will help disseminate these materials to educators across Virginia.

TECHNICAL REVIEW MATERIALS
DNA RESTRICTION ANALYSIS AND GEL ELECTROPHORESIS

Adrienne Warren
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1	What type of DNA are we using in our experiment?	circular plasmid DNA.
2	What restriction enzymes were used to digest the DNA?	EcoRI and/or PstI
3	What are restriction enzymes?	Restriction enzymes are enzymes that cleave double-stranded DNA at specific nucleotide sequences.
4	What is the recognition site for *Eco*RI and where is the cleavage point?	↓ 5’- G A A T T C -3’ 3’- C T T A A G -5’ ↑
5	What is the recognition site for *Pst*I and where is the cleavage point?	↓ 5’- C T G C A G -3’ 3’- G A C G T C -5’ ↑
6	How are the places that the restriction cleave enzymes (i.e., the enzyme recognition sites) symmetrical?	They are the same when read 5’ to 3’ on both strands. This is called a palindrome (e.g., level, 1881).
7	What are sticky ends?	When a restriction enzyme cuts the DNA, it can leave overhanging ends. These are sticky ends, which can rejoin with other cut ends.
8	Where do restriction enzymes come from? How are they named?	Restriction enzymes come from microorganisms such as *Escherichia coli and Haemophilus influenzae*. They are named for their organism of origin and the order in which they were discovered.
9	What function do restriction enzymes serve in bacterial cells?	They digest foreign (viral) DNA in order to protect the cell from invasion.
10	What is the purpose of electrophoresis?	To separate the DNA fragments by size.
11	What is electrophoresis?	A technique used to separate charged molecules in an electrical field.
12	What direction does a charged molecule move?	Toward the electrode of opposite charge.
13	What direction does DNA move? Why?	Toward the positive electrode (anode) because the DNA is negatively charged.
14	What function does the agarose serve?	Agarose acts as a sieve to separate the DNA molecules based on their size.
15	What is acrylamide used to separate?	Proteins and small DNA molecules (< 1000 basepairs).

16	What is agarose used to separate?	Most DNA molecules and some very large proteins.
17	What is agarose?	Agarose is a polysaccharide from seaweed (algae) that can be dissolved in hot water. It solidifies to a gelatin-like consistency when it cools.
18	What determines the size of the pores in the gel?	The concentration of agarose or acrylamide. Gels with a lower concentration of agarose will be more porous then gels with a higher concentration of agarose.
19	Larger molecules can be separated better with a more porous gel. Would you use a high or low concentration of agarose to get a more porous gel?	A lower concentration of agarose would mean more space between molecules, and therefore a more porous gel.
20	Why was a 0.9% agarose gel used in this experiment?	To separate mid-sized DNA fragments.
21	Why is a comb inserted into the gel before it solidifies?	To form wells in which to load the DNA.
22	Why is glycerol added to the DNA samples?	To make the DNA sample solution more dense (denser than the buffer) so that it will sink to the bottom of the well.
23	What function does Orange G dye serve?	Orange G dye is a tracking dye that migrates through the gel at the same rate as a 50 bp DNA molecule. It is used to estimate how far the DNA samples have migrated through the gel.
24	What is done when it is time to load and run the gel?	The gel is covered in buffer, the comb is removed, the samples are loaded in the wells, the lid is placed on the box, the gel box is connected to a power supply, and an electric current is passed through the gel.
25	Which DNA molecules move faster through the gel?	Smaller molecules move through the gel faster than large molecules do.
26	What function does ethidium bromide serve? Why are we not using it?	It stains the DNA so that it can be seen. It is a mutagen, and hazardous to handle.
27	What DNA stain will we use?	Methylene blue
28	What precautions must we take with DNA stains?	Wear gloves.
29	Why must the gel be destained?	The methylene blue soaks through the entire gel. We destain to remove the stain that is not bound to the DNA.
30	What is a DNA ladder?	A DNA ladder is a mixture of DNA fragments of known lengths. Using the data from the DNA ladder (migration distance of DNA fragments of known sizes), a standard curve can be constructed and used to calculate the sizes of unknown DNA fragments.
31	When making the gel, what two things must be combined?	Agarose and buffer.
32	Why should the agarose solution *not* be swirled before heating?	Some of the undissolved agarose might stick to the sides of the flask.
33	How is the agarose put into solution?	It is heated.
34	How do you know that the agarose solution is ready?	When the agarose is ready, i.e., in solution, it is clear.

35	Why is water added back to the heated agarose solution?	To make up for evaporation that occurred during heating.
36	What must be done to the agarose solution before it is poured into the casting trays?	It must cool to 55–60˚C.
37	How can you tell that the gel is ready?	It looks cloudy.
38	What do you do once the gel is ready?	Remove the gel from the casting tray, place it in the electrophoresis chamber, cover it with buffer, load the DNA samples, place the lid on the chamber, and connect the leads to the power source.
39	Describe how the gel box is to be connected to the power source.	Red to red and black to black.
40	How long should the gel be run?	Until the dye front nears or reaches the bottom of the gel.
44	What factors might affect the run times?	Gel volume, variations if buffer concentration, water used to mix buffer.
45	How would you get sharper bands of DNA?	Run the gel at a lower voltage.
46	After the gel has electrophoresed for the desired amount of time, what should be done?	The power supply should be turned off and the leads disconnected. The gel should be removed from the gel box and placed in a staining tray.
47	The gel is stained in what and for how long?	The gel should be stained in a 1x methylene blue solution for 15–20 minutes.
48	Describe how to destain the gel?	Pour off the stain and rinse the gel several times with water. Allow the gel to soak for several minutes in each change of water. Longer destaining times will result in reduced background staining.
49	What would happen if you destained the gel in large volumes of water?	If too much water is used to destain the gel, the stained DNA fragments can be washed out of the gel. The water used to destain should never be deeper than just enough to cover the gel.
50	What is used to view the stained DNA bands?	A light box.

MAKING A DILUTE BUFFER SOLUTION

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Example: Using a 20x buffer and diluting to a 1x buffer would be a ratio of

1:20 (1 part buffer to 20 parts total)

A final volume of 300 ml would be made by first dividing by the total number of parts:
$300 \div 20 = 15$

Based on this, 15 ml of 20x buffer would be added to enough water to reach the final volume of 1x buffer wanted:

15 ml buffer + 285 ml water = 300 ml 1x buffer

Exercises:

1. Calculate the amount of 20x buffer and the amount of water needed to make 500 ml of a 1x buffer solution.

2. Calculate the amount of 50x buffer and the amount of water needed to make 400 ml of a 5x buffer solution.

3. Calculate the amount of 20x buffer and the amount of water needed to make 500 ml of a 10x buffer solution.

4. Calculate the amount of 10x buffer and the amount of water needed to make 300 ml of a 1x buffer solution.

Answers:

Question #	Buffer volume	Water volume
1	25 ml	475 ml
2	40 ml	360 ml
3	250 ml	250 ml
4	30 ml	270 ml

MAKING AGAROSE GELS

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Example: To make a 1% gel, use 1 gram (g) of agarose and 100 milliliters (ml) of 1x buffer solution:

1% = 1 g/100 ml

Based on this, a general formula is:

% x ml = g

The gel percentage must be in decimal form, in other words, 1% = 0.01, 0.6% = 0.006, and 3% = 0.03. So, for example, to make a 2% gel in 50 ml of buffer, you would need to add 1 g of agarose. [(0.02) (50) = 1]

Exercises: Tell the amount of the missing component required to make the following gels.

1. Make a 2% gel using 100 ml of buffer.
2. Make a 2% gel using 200 ml of buffer.
3. Make a 0.5% gel using 50 ml of buffer.
4. Make a 0.9% gel using 50 ml of buffer.
5. Make a 1% gel using 3 g of agarose.
6. Make a 4% gel using 2 g of agarose.
7. Make a 0.5% gel using 1 g of agarose.
8. Make a 0.8% gel using 0.8 g of agarose.
9. What % gel is made by using 1 g of agarose and 200 ml of buffer?
10. What % gel is made by using 2 g of agarose and 300 ml of buffer?
11. What % gel is made by using 0.7 g of agarose and 100 ml of buffer?
12. What % gel is made by using 0.5 g of agarose and 80 ml of buffer?
13. Using 2.9 g of agarose, how much buffer do you need to make a 0.9% gel?

Answers:

Question #	Answer
1	2 g agarose
2	4 g agarose
3	0.25 g agarose
4	0.45 g agarose
5	300 ml buffer
6	50 ml buffer
7	200 ml buffer
8	100 ml buffer
9	0.5%
10	0.67%
11	0.7%
12	0.625%
13	322 ml of buffer

AGAROSE GEL QUIZ

Adrienne Warren, Chesapeake Center for Science and Technology

1. Before an agarose gel can be made, buffer must usually be diluted to a 1x concentration. Given a 20x concentrated buffer solution, how would you make 400 ml of a 1x buffer solution?
2. Given a 20x concentrated buffer solution, how would you make 1 liter of a 1x buffer solution?
3. Given a 50x concentrated buffer solution, how would you make 800 ml of a 5x buffer solution?
4. Once the 1x buffer is made, the agarose gel can be made. How many grams of agarose are needed to make 300 ml of a 1% gel?
5. How many grams of agarose are needed to make 500 ml of a 2% gel?
6. How many grams of agarose are needed to make 400 ml of a 0.5% gel?
7. How many milliliters of buffer are needed to make a 1% gel if you are using 2 grams of agarose?
8. How many milliliters of buffer are needed to make a 3% gel if you are using 6 grams of agarose?
9. How many milliliters of buffer are needed to make a 0.8% gel if you are using 4 grams of agarose?
10. What % gel would you make if you combined 2 grams of agarose with 200 ml of buffer?
11. What % gel would you make if you combined 5 g of agarose with 1 liter of buffer?
12. What % gel would you make if you combined 3.6 grams of agarose with 400 ml of buffer?

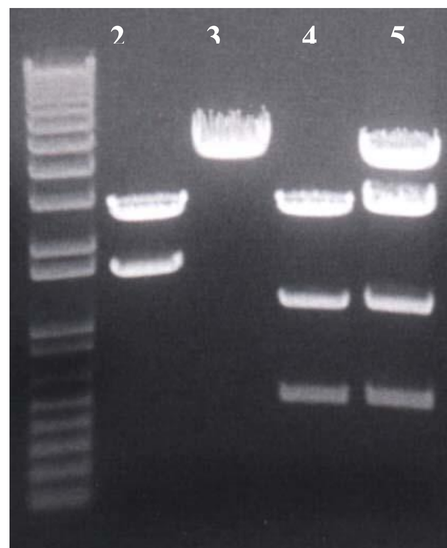
Answers:

1. Buffer: 20 ml
   Water: 380 ml
2. Buffer: 50 ml
   Water: 950 ml
3. Buffer: 80 ml
   Water: 720 ml
4. 3 grams
5. 10 grams
6. 2 grams
7. 200 ml
8. 200 ml
9. 500 ml
10. 1%
11. 0.5%
12. 0.9%

Teacher Guide for Scenarios

These are the samples that should be used in the scenarios.

Lane 1 DNA ladder
Lane 2 EcoRI digest
Lane 3 PstI digest
Lane 4 EcoRI & PstI double digest
Lane 5 Crime scene sample



Cystic Fibrosis Scenario

DNA	lid color**	In scenario
EcoRI-cut plasmid	yellow	Tom's DNA
Pst1-cut plasmid	green	Susan's DNA
Mixture of Pst1-cut & double digest	red	DNA of the four most common cystic fibrosis alleles

In this scenario, you do not need the double digest sample.

Mystery of the Stolen Cat Food

DNA	lid color**	In scenario
EcoRI-cut plasmid	yellow	Cat suspect 1
Pst1-cut plasmid	green	Cat suspect 2
Double digest with EcoRI + PstI	blue	Fluffy
Mixture of Pst1-cut & double digest	red	Fluffy's bowl

In this scenario, the DNA in the food bowl will be a mixture DNA from Suspect cat 2 and Fluffy.

DNA Detectives Or "Who Dunnit?"

DNA	lid color**	In scenario
EcoRI-cut plasmid	yellow	Suspect X
Pst1-cut plasmid	green	Suspect Y
Double digest with EcoRI + PstI	blue	Suspect Z
Mixture of Pst1-cut & double digest	red	crime scene

In this scenario, the DNA at the crime scene will be a mixture DNA from Suspects Y and Z.

** These are the lid colors on the tubes as shipped from Fralin.

Teacher Guide: Cystic Fibrosis Scenario and Ethical Discussion

This teacher guide is provided to give sample answers to questions. Most of the questions are open-ended, so students may have correct answers that aren't included in this guide. Although the experiment is set up to yield one correct answer, there are variations in data among students. As long as students examine their data carefully and can justify their answers based on their data, that's science! Data are always right and there isn't necessarily a ”right answer.”

The Scenario:

Susan and Tom have been married for three years and are considering having children. The disease cystic fibrosis runs in Tom's family and so the couple decide to get tested to see if either of them has the version (allele) of the CF gene that is correlated with the disease. Because cystic fibrosis is inherited in an autosomal recessive pattern (the affected gene is on chromosome 7, and two defective alleles are necessary for a person to develop the disease), Tom and Susan could each carry one defective allele that they could pass on to their children.

Approximately 900 different mutations, or DNA changes that result in defective alleles, have been identified. In this activity, students will examine Tom's and Susan's alleles and compare them against the four most common alleles. In this scenario, the 4,200 bp allele is normal, and the other alleles (2,700, 1,100, and 400 bp) are defective. Each individual may have two of the same alleles or two different alleles.

Materials:

DNA ladder
agarose
TAE buffer
10 µl fixed volume pipettors
DNA from Tom (EcoRI digest)
DNA from Susan (PstI digest)
DNA from the four most common alleles for comparison (mix of PstI digest and double-digest)
yellow pipette tips
electrophoresis apparatus
power supply

Procedure:

1. Prepare an agarose gel as instructed by your teacher. Don't touch/move your gel until it's hard. **Why not?**

   If the agarose moves while it's hardening, it will harden unevenly, making it more difficult for the DNA to move through evenly.

   Draw a picture of your gel and label which samples are where before you add DNA to the gel.

   The samples are indistinguishable once they are loaded, so everyone needs to have a drawing of what they will load where before they load their DNA samples. This drawing will be useful during the analysis, once the gels are stained.

2. Pour TAE solution over your gel CAREFULLY so that is it completely covered plus a little more. **What do you think the TAE solution is for?**

   TAE is like saltwater - it conducts electricity, plus it is a buffer to control for pH changes.

3. Load your **DNA SAMPLES** into the wells near the **BLACK ELECTRODE**. **Why near the black electrode?** Be sure to keep track of which samples you loaded in which lanes.

   DNA is negatively charged, so to move the DNA into the gel with electricity, the DNA needs to be loaded on the negative or black side, it will then move towards the red. If it's loaded near the red electrode, it will migrate off the gel into the TAE buffer.

4. Run that gel!! Plug the electrodes into your gel box (**red to red, black to black**), being careful not to bump your gel too much. Plug the power source into an outlet. **How can you tell your gel is running?**

   It bubbles at the electrodes. This is a redox reaction, forming H_2 gas at the black electrode and O_2 gas at the positive electrode.

Analyzing Your DNA Data

Run the gels until the orange dye is approximately 1 cm from the bottom of the gel (about 15–20 minutes).

The orange samples will move to the right (towards the positive red electrode). They move to the positive electrode because they are negatively charged. The DNA cannot be observed until the gel is stained because DNA is colorless. We add orange coloring to the DNA samples so you can see the samples as you are loading them. The orange samples will continue to travel toward the positive electrode and separate farther. Run the gels until the orange travels 2/3 of the way across the gel (about 15–20 minutes).

Place the gels carefully in staining trays. Pour methylene blue to cover the gel. Let the stain sit on the gel for about 15–20 minutes. Pour the stain back in the bottle and gently rinse your gel with water. Allowing the gel to soak in water for about 20 minutes will make the bands more pronounced.

Place the gels on light boxes to examine the alleles. Draw a picture.

Remind students that the 4,200 bp band is the normal allele. Students should determine whether Tom and Susan have normal or defective alleles by comparing with the four most common alleles.

What can your data tell you about Tom's and Susan's chances of having a child with cystic fibrosis?

Students should have an answer based on their data (e.g. I think Tom and Susan have no chance of having a child with cystic fibrosis because only one of them has a defective allele and a child must inherit two defective alleles to develop the disease; or The data aren't clear, we can't line up Tom's and Susan's alleles with any of the four most common alleles, we would have to redo the experiment to be sure.).

If you're dying to know whether either Tom or Susan have any defective alleles, Tom has one defective allele (2,700 bp) and one allele (1,500 bp) that doesn't line up with any of the four most common alleles. This allele is not defective because we know that Tom doesn't have the disease, so it must be an uncommon but functional allele. Susan has no defective alleles, thus their children will not develop cystic fibrosis.

However, no one tells a genetic testing service what answer they are supposed to find when they perform a genetic test. So feel free to NEVER tell your students the "answer" — they have to be confident about what their data show.

Name: ________________________
Period: _____

Cystic Fibrosis Scenario

The Scenario:

Susan and Tom have been married for three years and are considering having children. The disease cystic fibrosis runs in Tom's family and so the couple decide to get tested to see if either of them has the version (allele) of the CF gene that is correlated with the disease. Because cystic fibrosis is inherited in an autosomal recessive pattern (the affected gene is on chromosome 7, and two defective alleles are necessary for a person to develop the disease), Tom and Susan could each carry one defective allele that they could pass on to their children.

Approximately 900 different mutations, or DNA changes that result in defective alleles, have been identified. In this activity, you will examine Tom's and Susan's alleles and compare them against the four most common alleles. In this scenario, the 4,200 base pair allele is normal, and the other alleles (2,700, 1,100, and 400 base pairs) are defective. Each individual may have two of the same or two different alleles.

Materials:

agarose
TAE buffer
10 µl fixed volume pipettors
yellow pipette tips
electrophoresis apparatus
DNA from four most common alleles for comparison
power supply
DNA ladder
DNA from Tom
DNA from Susan

Procedure:

1. Prepare an agarose gel as instructed by your teacher. Don't touch/move your gel until it's hard. **Why not?**

Draw a picture of your gel and label which samples are where before you add DNA to the gel.

2. Pour TAE solution over your gel CAREFULLY so that is it completely covered plus a little more. **What do you think the TAE solution is for?**

3. Load your **DNA SAMPLES** into the wells near the **BLACK ELECTRODE**. **Why near the black electrode?** Be sure to keep track of which samples you loaded in which lanes.

4. Run that gel!! Plug the electrodes into your gel box (**red to red, black to black**), being careful not to bump your gel too much. Plug the power source into an outlet. **How can you tell your gel is running?**

Analyzing Your DNA Data

Run your gel until the orange dye is approximately 1 cm from the bottom of the gel (about 15–20 minutes). Place your gel carefully in a staining tray. Pour methylene blue to cover the gel. Let the stain sit on the gel for about 15–20 minutes. Pour the stain back in the bottle and gently rinse your gel with water.

Place the gels on light boxes to examine the alleles. Draw a picture.

What can your data tell you about Tom's and Susan's chances of having a child with cystic fibrosis?

References

Genetics Home Reference: Information about Cystic Fibrosis
http://ghr.nlm.nih.gov/condition/cystic-fibrosis
Online Mendelian Inheritance in Man (OMIM): Information about Cystic Fibrosis
http://omim.org/entry/219700

Cystic Fibrosis Scenario: Ethical Discussion of Genetic Testing

The Institute of Practical Ethics and Public Life at the University of Virginia has developed a framework for guiding ethical discussions in biotechnology (*Bioethics and Biotechnology: An Executive Education Program* © Johns Hopkins Berman Bioethics Institute, University of Virginia Institute of Practical Ethics, and Georgetown University Kennedy Institute of Ethics and Law Center, 2002). The Institute has given us permission to adapt these materials to guide students in considering the ethics of biotechnology, especially as it relates to genetic testing.

When people are considering having a genetic test done, there are a host of questions they must consider with the help of a genetic counselor. For example:

1. Cystic fibrosis runs in Tom's family, but no one in Susan's family has had cystic fibrosis. Yet, if Susan gets tested and is found to have a defective allele, she is inadvertently testing her parents and siblings. If she has a defective allele, then she has information that suggests that one of her parents has a defective allele and her siblings might have it as well. If she finds out that she has a defective allele, should she tell her family or keep the results private?
2. Some diseases, like heart disease, are caused by a combination of genetic and environmental factors. For example, if a person has an allele that predisposes them to develop heart disease, but eating fatty foods would greatly increase their chances of developing heart disease, should they have the test done so that they can choose safer behaviors, like eating a low fat diet?
3. Some diseases are caused by defective alleles and genetic tests can be done to determine whether an individual will develop the disease, yet there is no known treatment for the disease (e.g., Huntington's Disease). If no treatments are available, should an individual have a genetic test done?

The purpose of an ethical analysis is to encourage good, structured thinking. Ethical analysis helps the discussants to:

- Understand the context.
- Throw the net wide.
- Consider full effects.
- Recognize limitations.
- Use principles and imagination to resolve or minimize tensions.

Ethical analysis should be continuous, individual, and social. Sometimes it results in better solutions. Sometimes it reveals tension that can be resolved by principles. Sometimes imagination can transcend the tension. Sometimes it causes us to question the principles we hold.

Ethical analysis is best practiced in group discussions that involve:

- Listening
- Understanding others' perspectives
- Expressing values and opinions
- Thinking independently

One Approach to Ethical Analysis

After completing the genetic testing activity, challenge your students to consider some of the questions outlined above using the following approach. This is a simple framework for ethical analysis. It is not a decision-making procedure. It will not automatically produce ethical answers. However, the framework will encourage participants to consider all of the ethical aspects of a particular issue. Hopefully, it will also stimulate conversation and debate.

The framework should be used to identify the critical ethical issues in a case, explore alternative actions, and encourage participants to make a decision that they can defend ethically in accordance with their beliefs. Achieving consensus is not the ultimate goal.

Apply the framework by addressing the following questions:

1. What are the facts of the situation?
2. What are the initial options for action?
3. What is at stake in the situation and in alternative courses of action?
4. Who are the critical stakeholders?
5. Are there any practical constraints (e.g., legal or institutional constraints like laws, policies, or regulations)?
6. Are there any creative alternatives?
7. Which action should be taken, all things considered?

Presenter's or moderator's role:

1. Listen well.
2. Ask good questions.
3. Set ground rules for case discussion. For example:
    a. Everyone should try to participate.
    b. It is an opportunity to experiment. People will not be held to their views afterward. People can even take contrarian positions (play devil's advocate), just for the sake of argument.
    c. The discussion should be fun. People should not hesitate to respond to other comments or to share responses based on personal feelings.
    d. Unpopular views are welcome.
    e. Everyone is not expected to come to the same conclusion, but everyone is expected to listen and treat others' views with respect.
4. Encourage everyone to participate. This might mean calling on students who have not raised their hands.
5. During the last 10–15 minutes of discussion, summarize the discussion and refer back to conceptual materials introduced earlier, including the science content and the ethical framework. It is often good to end by noting that there are no easy answers and that ethical analysis is a way of thinking that can help individuals incorporate ethical values into their interactions through discussion with their peers.

Teacher Guide: Mystery of the Stolen Cat Food

This teacher guide is provided to give sample answers to questions. Most of the questions are open-ended, so students may have correct answers that aren't included in this guide. Although the experiment is set up to yield one correct answer, there are variations in data among students. As long as students examine their data carefully and can justify their answers based on their data, that's science! Data are always right and there isn't necessarily a 'right answer'.

The Scenario:

Fluffy is my white haired cat. She sometimes likes to have her meals on the back porch, so I put her dish of cat food outside. Recently she's been whining and complaining because she's hungry. After doing a little detective work, I realized that another white haired cat has been eating Fluffy's food. However, there are 2 other white haired cats in my neighborhood and I can't tell which cat is eating Fluffy's food. You are going to help me figure out which cat is the criminal!

Some questions to get you thinking about today's lab:

What is DNA and what does it do?

DNA is deoxyribonucleic acid, and it makes you who you are, codes for proteins, serves as the basis for heredity, etc.

What are some characteristics or properties of DNA?

double-helix, double stranded, negatively charged, colorless, unique sequence in each individual

How can we take advantage of these properties to help us figure out which cat is eating Fluffy's food?

We can take DNA from different individuals and cut it with restriction enzymes. Because each individual has a unique DNA sequence, the restriction enzymes will cut the DNA into different sizes for different individuals. We can take advantage of the negative charge of DNA to separate out the different sized pieces to determine whose pieces match the pieces at the crime scene. With younger students, you may choose to just focus on the idea that everyone's DNA is different (except identical twins!) so everyone will have different sized pieces of DNA.

What tricks can we use to see DNA?

DNA stain such as methylene blue

Materials
DNA from cat suspect #1, cat suspect #2, Fluffy (#3), and the cat dish (X)
DNA ladder
agarose
TAE buffer
10 µl fixed volume pipettors
yellow pipette tips
electrophoresis apparatus
power supply

Procedure:

1. Prepare an agarose gel as instructed by your teacher. Don't touch/move your gel until it's hard. **Why not?**

   If the agarose moves while it's hardening, it will harden unevenly, making it more difficult for the DNA to move through evenly.

   Draw a picture of your gel and label which samples are where before you add DNA to the gel.

   The samples are indistinguishable once they are loaded, so everyone needs to have a drawing of what they will load where before they load their DNA samples. This drawing will be useful during the analysis, once the gels are stained.

2. Pour TAE solution over your gel CAREFULLY so that is it completely covered plus a little more. **What do you think the TAE solution is for?**

   TAE is like saltwater - it conducts electricity, plus it is a buffer to control for pH changes.

3. Load your **DNA SAMPLES** into the wells near the **BLACK ELECTRODE**. **Why near the black electrode?** Be sure to keep track of which samples you loaded in which lanes.

   DNA is negatively charged, so to move the DNA into the gel with electricity, the DNA needs to be loaded on the negative or black side, it will then move towards the red. If it's loaded near the red electrode, it will migrate off the gel into the TAE buffer.

4. Run that gel!! Plug the electrodes into your gel box (**red to red, black to black**), being careful not to bump your gel too much. Plug the power source into an outlet. **How can you tell your gel is running?**

   It bubbles at the electrodes. This is a redox reaction, forming H_2 gas at the black electrode and O_2 gas at the positive electrode.

Analyzing Your DNA Data

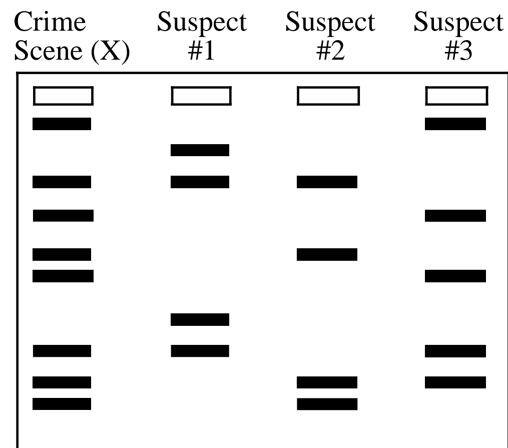
Start your gel running and after **5 minutes** draw another picture.

The orange samples will move to the right (towards the positive red electrode). They move to the positive electrode because they are negatively charged.

The DNA cannot be observed until the gel is stained because DNA is colorless. We add orange coloring to the DNA samples so you can see the samples as you are loading them. The orange samples will continue to travel toward the positive electrode and separate farther. Run the gels until the orange travels 2/3 of the way across the gel (about 15–20 minutes).

Place your gel carefully in a staining tray. Pour methylene blue to cover the gel. Let the stain sit on the gel for about 15–20 minutes. Pour the stain back in the bottle and gently rinse your gel with water. Place your gel directly on a light box, what does it look like? Draw a picture.

Here is a sample gel. Students need to identify which suspect's DNA bands match those from the crime scene.



Sample DNA fingerprint

What can your data tell you about which cat suspect committed the crime?

Students should have an answer based on their data (e.g. I think it's suspect #2 because the crime scene DNA pieces line up with suspect #2's DNA pieces; The data aren't clear, we can't line up the crime scene DNA pieces with any of the potential suspects, we would have to redo the experiment to be sure.). If you're dying to know whose DNA is at the crime scene, it's #2 and 3. But, no one tells a jury whether a murderer is guilty, the jurors have to look at the data and decide. So feel free to NEVER tell your students the 'answer' — they have to be confident about what their data show.

Name: ________________________
Period: _____

Mystery of the Stolen Cat Food

The Scenario:

Fluffy is my white haired cat. She sometimes likes to have her meals on the back porch, so I put her dish of cat food outside. Recently she's been whining and complaining because she's hungry. After doing a little detective work, I realized that another white haired cat has been eating Fluffy's food. However, there are 2 other white haired cats in my neighborhood and I can't tell which cat is eating Fluffy's food. You are going to help me figure out which cat is the criminal!

Some questions to get you thinking about today's lab:

What is DNA and what does it do?

What are some characteristics or properties of DNA?

How can we take advantage of these properties to help us figure out which cat is eating Fluffy's food?

What tricks can we use to see DNA?

Materials
DNA from cat suspect #1, cat suspect #2, Fluffy (#3), and the cat dish (X)
DNA ladder
agarose
TAE buffer
10 µl fixed volume pipettors
yellow pipette tips
electrophoresis apparatus
power supply

Procedure:

1. Prepare an agarose gel as instructed by your teacher. Don't touch/move your gel until it's hard. **Why not?**

   Draw a picture of your gel and label which samples are where before you add DNA to the gel.

2. Pour TAE solution over your gel CAREFULLY so that is it completely covered plus a little more. **What do you think the TAE solution is for?**

3. Load your **DNA SAMPLES** into the wells near the **BLACK ELECTRODE**. **Why near the black electrode?** Be sure to keep track of which samples you loaded in which lanes.

4. Run that gel!! Plug the electrodes into your gel box (**red to red, black to black**), being careful not to bump your gel too much. Plug the power source into an outlet. **How can you tell your gel is running?**

Analyzing Your DNA Data

Start your gel running and after **5 minutes** draw another picture.

Place your gel carefully in a staining tray. Pour methylene blue to cover the gel. Let the stain sit on the gel for about 15-20 minutes. Pour the stain back in the bottle and gently rinse your gel with water. Place your gel directly on a light box, what does it look like? Draw a picture.

What can your data tell you about which cat suspect committed the crime?

DNA DETECTIVES OR "WHO DUNNIT?"

Pat Neeley, Jefferson Forest High School, Forest, VA

Introduction: Many of the revolutionary changes that have occurred in biology over the past fifteen years can be attributed to the ability to manipulate DNA in defined ways. The principal tools for the recombinant DNA technology are enzymes that can "cut and paste" DNA. Restriction enzymes are the "chemical scissors" of the molecular biologist; these enzymes cut DNA at specific nucleotide sequences. A sample of someone's DNA, incubated with restriction enzymes, is reduced to millions of DNA fragments of varying sizes. A DNA sample from a different person would have a different nucleotide sequence and would thus be enzymatically "chopped up" into a very different collection of fragments. Because no two people (except identical twins) have exactly the same DNA, a person's DNA fingerprint is unique and can be used for the purposes of identification. We have been asked to apply DNA fingerprinting to determine which suspect should be charged with a crime perpetrated in our city.

WHO DUNNIT?

A murder has been committed, and police discover evidence of a struggle and blood traces at the scene of the crime. Ian, a UPS deliveryman, is found dead in his truck on Rt. 221 just west of Forest Middle School. Autopsy has shown that Ian was strangled to death, but there is no blood from the victim on the scene. Packages are missing from the truck, and no witnesses can be found. Suspects X, Y, and Z are arrested and will go through DNA tests to determine if they were at the scene of the crime. ***What DNA sources other than blood might be found at the crime scene?*** All of the suspects proclaim their innocence adamantly, and all want to see their lawyers. At their indictments, it is learned that:

- Suspect X - Bob Smith is a man in his middle thirties with prior convictions for armed robbery. Bob was apprehended shortly after the murder in Bedford driving recklessly on an expired license. No contraband was found in his possession but his hands are cut in several places. He says it's because he works construction.
- Suspect Y - Jim Dale is a man in his late forties. He is suspected of being romantically involved with Ian's wife, Pam. Unexplained scratches were found on the back of his neck.
- Suspect Z - Pam, wife of Ian. She says she was with Jim the entire day. Several cuts on both hands are suspicious. She claims she got them while picking blackberries with Ian.

You are the lab worker who has been handed the DNA samples from the three suspects involved, plus the DNA from the blood at the crime scene. Using molecular biology techniques, your job is to determine which of the suspects might have been at the crime scene. The court awaits your findings.

Purpose: To prepare and analyze a DNA fingerprint, the student will:

1. Prepare and load an agarose gel with enzyme-cut DNA samples.
2. Conduct gel electrophoresis to sort out the DNA fragments in the samples.
3. Stain the gel to visualize the DNA fragments.
4. Analyze the resulting banding pattern or "DNA fingerprint" to solve a crime.

Materials:

- electrophoresis chamber
- casting tray
- comb
- agarose solution
- power supply
- plastic tray for storing gel
- DNA from suspects and crime scene
- micropipette and pipette tips
- electrophoresis buffer (0.25x TAE)
- racks for 1.5 ml microcentrifuge tubes
- 1.5-ml microcentrifuge tubes
- staining solution
- gloves
- light box
- plastic wrap

Procedures:

1. Prepare the casting tray by inserting the metal buffer dams. Insert the comb into the tray.
2. Weigh the flask containing the agarose solution and heat the flask until the agarose is completely into solution. Cool flask briefly, then reweigh it.
3. Slowly add distilled water to the flask until the total mass equals the mass you started with. (This will replace any water last during the heating and prevent your gel from becoming too dense.)
4. After the gel solution has cooled to 50–60˚C, carefully pour the agarose into the casting tray. DO NOT jar or move the casting tray as the gel solidifies. As the agarose sets or gels, it changes from clear to slightly opaque.
5. After the gel has set, carefully remove the comb and casting gates.
6. Pour enough buffer into the gel box so that the gel is completely covered, with no “dimpling” above the wells.
7. Load the entire contents (10 µl) of each sample tube into separate wells in the gel. Be sure that the micropipettor tip is below the surface of the buffer and just above the center of each well that you load. CHANGE PIPETTE TIPS BETWEEN SAMPLES TO AVOID CONTAMINATION! Leaving an empty lane on both sides, load in the order shown below:

   X **Y** **Z** **E** **L**

   X = Bob Smith, former thief
   Y = Jim Dale, boyfriend
   Z = Pam, wife of victim
   E = evidence DNA found at crime scene
   L = ladder DNA (standardized control sample)

8. Once the wells are loaded, put the top on the gel box and connect it to the power supply. Plug in the power supply and turn the unit to the desired voltage. Run until the loading dye nears of the bottom of the gel. At this point, the current can be turned off and the leads (wires) disconnected.

   The term “electrophoresis’ literally means “to carry with electricity.” It is a technique for separating and analyzing mixtures of charged molecules. When placed in an electric field, pieces of DNA (because they are ionized and negatively charged) migrate toward the positive electrode (anode); small pieces of DNA experience less resistance and move faster (farther) than larger pieces.

9. Remove the casting tray from the gel box. **Carefully** slide your gel off the casting tray and into its plastic container.
10. Wearing gloves, pour the methylene blue staining solution into the plastic container and allow it to sit for 15–20 minutes, rocking the container periodically.
11. Pour the stain carefully back into the beaker (do not throw away) and gently rinse your gel with water for 5 minutes. You may need to let your gel soak in several changes of water to increase contrast. **Do not** use large volumes of water. The water should just cover the gel. It is better to use small volumes of water and change it frequently than to flood the gel in a large volume!
12. Place a piece of plastic wrap on your light box and examine your stained, rinsed gel by placing it on the plastic wrap. Gently place a transparency over your gel and trace the bands with a permanent marker.
13. Store your gel in a labeled plastic bag in the refrigerator.

Upon completion of the lab

- dispose of designated materials in the appropriate places.
- leave equipment as you found it.
- check that your work station is in order.
- wash your hands.

Analysis:

Compare the fingerprints of all the suspects in this case to the DNA profile of the DNA isolated from the blood droplets at the crime scene. By comparing the banding patterns of the DNA samples you should be able to determine who the murderer was. Which suspect's blood was found at the site of the murder?

Unfortunately, most courts will not accept your preliminary results as being conclusive and will expect a more detailed analysis.

DNA is made up of a series of base pairs (guanine-cytosine, adenine-thymine, cytosine-guanine, thymine-adenine).

G - C
A - T
C - G
T - A

Every individual has a unique series of base pairs in their DNA.

The DNA samples that were used had been treated with a restriction enzyme which seeks out specific DNA base pair (bp) sequences and cuts the DNA at that point. Since the DNA samples were all different, they were all cut at different spots, which resulted in different size pieces of DNA for each sample.

A T C C T G C | C G G A A G T C C G A T C | C G G T A
T A G G A C G G C | C T T C A G G C T A G G C | C A T

After the DNA is cut, the restriction fragments are separated from each other by using electrophoresis. Electrophoresis will separate fragments based on their size and charge. The phosphate groups, which

make up part of the backbone of the DNA, carry a negative charge, so they will move through the gel toward the positive pole (red). The smaller fragments will move faster than the larger ones. After staining, the bands of DNA which represent different size fragments, can be analyzed. By calculating the size of the fragments from different samples, it is possible to determine guilt more definitively.

Analysis Procedure

1. First, look at the lane containing the 1 kb (kilobase) DNA ladder. To figure out which band is which, find the two bands that represent 1650 and 2000 base pairs (bp). These two bands are separated from the other bands and are rather easy to find.

2. Find these two bands on the stained gel and measure their migration from their point of origin in the gel. Measure from the bottom of the well to the foremost edge of the stained band. Be certain to measure each from the same point, e.g., from the bottom of the well each time, not the bottom one time and the top on the well the next. Record the base pair size of the band and it's migration distance.

3. Working up (toward larger DNA fragments) and down (toward smaller DNA fragments) from the 1650 and 2000 bp bands, record the migration of the other bands in the DNA ladder.

4. Measure the migration of the bands in the experimental lanes and record the migration distances.

5. Create a standard curve using the data from the 1 kb DNA ladder. Graph the migration distance of the DNA fragments (x-axis) against the size of the DNA fragments (y-axis) on semi-log graph paper.

6. Draw a straight "line of best fit," which comes as close as possible to each point.

7. To determine the size of an enzyme-digested DNA fragment, find where the migration distance of the DNA fragment intersects the standard curve. Draw a line from this point to the y-axis. Where this line meets the y-axis is the size of the fragment.

8. Using your calculated bp values, determine which suspect should be charged with the crime.

STATE AND NATIONAL SCIENCE EDUCATION STANDARDS: WHERE DOES BIOTECHNOLOGY FIT?

Source: National Science Education Standards

The National Science Education Standards (NSES) are guidelines developed by a team of teachers, university-based science educators, scientists, and science education policymakers. This text is available for purchase [National Science Education Standards. (1996) National Research Council. National Academy Press, Washington, D.C. ISBN 0-309-05326-9] or as downloadable PDF files of individual pages and chapters at:
http://books.nap.edu/html/nses/html/index.html

- DNA is the instructions for specifying characteristics, DNA is a large polymer formed from four kinds of subunits (A, T, C, G) with specific chemical and structural properties, genetic information underlies heredity coded in genes and replicated by a templating mechanism, each DNA molecules forms a single chromosome
- Most cells in a human contain two copies of each of 22 chromosomes, there is also a pair of sex chromosomes (X and Y), transmission of this information to offspring occurs through egg and sperm cells that contain only one representative from each chromosome pair, egg and sperm unite to form a unique individual
- Changes in DNA, or mutations, occur spontaneously at low rates, some of these changes make no difference to the organism, whereas others change cells and organisms

Source: Project 2061 (including *Science for All Americans* and *Benchmarks for Science Literacy*)

Project 2061 is a long-term initiative of the American Association for the Advancement of Science (AAAS) to help improve K-12 education so that all high school graduates are literate in science, math, and technology. More information can be found at: http://www.project2061.org

- Offspring are very much like their parents but still show some variation
- Over many generations, these differences can accumulate, so organisms can be very different from their ancestors
- Instructions for development are passed from parents to offspring in thousands of discrete genes, or segments of a molecule of DNA
- Sorting and combination of genes in sexual reproduction results in a great variety in the gene combinations of two parents
- Mixing of genes is not the only source of variation, occasionally some of the information in a cell's DNA is altered (deletions, insertions, substitutions), sometimes yielding new characteristics in offspring that may help organisms that have it to thrive and reproduce, or reduce that ability, or have no appreciable effect

Source: Virginia Standards of Learning

Up-to-date website outlining standards:
http://www.pen.k12.va.us/testing/sol/standards_docs/science/index.shtml
To help in thinking about how biotechnology fits into the Virginia State Standards, we have listed possible applicable standards below (adapted from the website). To see a complete list of standards, please refer to the website.

Overview of the Virginia Biology Standards of Learning Sample Scope and Sequence

Organizing Topics	Related Standards of Learning
Chemistry Concepts Important to the Study of Biology	BIO.1 a, b, c, e, f, i; BIO.3 a, b, c
Cell Theory	BIO.1 a; BIO.2 a, b, c, e; BIO.4 a, b, c, d; BIO.5 f; BIO.6 a, b, c
Genetics	BIO.1 b, d, e, f, g; BIO.2 a, b, c, d, e; BIO.6 d, e, f, g
Natural Selection	BIO.1 d; BIO.7 a, b, c, d, e, f; BIO.8 a, b, c, d
Life Functions and Processes	BIO.1 a, b, d, i; BIO.2 c; BIO.3 d; BIO.5 d, e
Ecology	BIO.1 a, d, h; BIO.5 a, b, c, g; Bio. 7a, g; BIO.9 a, b, c, d, e

Organizing Topic	Essential Knowledge and Skills	Related SOL
Chemistry Concepts Important to the Study of Biology	Recognize that the pH of pure water is 7, but that various substances can lower or raise the pH. A solution with pH below 7 is acidic. A solution with a pH above 7 is basic.	BIO.3 a
	Apply the following principles of scientific investigation: 1. Identify variables that must be held constant. 2. Identify the independent variable in an experiment. 3. Select dependent variables that allow collection of quantitative data. 4. Collect preliminary observations. 5. Make clear distinctions among observations, inferences, and predictions. 6. Formulate hypotheses based on cause and effect relationships. 7. Use probeware for data collection.	Bio.1 a, b, c, i
	Summarize DNA and RNA structure and function to include the following: 3. Genetic code is a sequence of DNA nucleotides. 4. The sequence of the DNA nucleotides is the genetic code. 5. The DNA code must be transcribed to messenger RNA in order for cells to make protein. 6. At the ribosome, amino acids are linked to form specific proteins. The sequence is determined by the mRNA molecule.	

Organizing Topic	Essential Knowledge and Skills	Related SOL
Genetics	Outline the major historical steps in determining DNA structure, including: • Genetic information encoded in the DNA molecules provides instructions for assembling protein molecules. The code is the same for all life forms.	BIO.1 b BIO.2 a, b, c, d, e
	Given a DNA sequence, write a complementary mRNA strand (A-U, T-A, C-G and G-C).	BIO.6 f
	Explain that DNA technologies allow scientists to identify, study, and modify genes. Forensic identification is one example of the application of DNA technology.	BIO.6 g
	Recognize that genetic engineering techniques provide great potential for useful products (e.g., human growth hormone, insulin, and resistant fruits and vegetables).	BIO.6 g
	Identify the Human Genome Project as a collaborative effort to map the entire gene sequence. This information will be useful in detection, prevention, and treatment of many genetic diseases. It also raises practical and ethical questions.	BIO.6 g
	Summarize possible results of genetic recombination: • Inserting, deleting, or substituting DNA segments can alter genes.	BIO.6 g

SOURCES FOR MATERIALS AND EQUIPMENT INCLUDED IN THE DNA BIOTECHNOLOGY KIT

Listed here are the sources for the equipment in the kits, as well as some alternative sources for the same (or similar) equipment. This is by no means an exhaustive list of the alternatives, just a few for pricing comparisons.

Gel boxes: The Bio-Rad gel boxes are $281.00 (catalog #166-4270EDU). Replacement casting gates are $59.50 per pair, combs $37.10 each, and gel trays $36.40 each.

Power supplies: The Bio-Rad PowerPac Basic power supplies are $324.00 (catalog #164-5050EDU).

Microcentrifuges: The personal microcentrifuges are available from Bio-Rad for $299.00 (catalog #166-0603EDU) and Edvotek for $279 (catalog #534).

Light boxes: The Mini-Pro light boxes are no longer available from Carolina Biological. They have a replacement product (larger) that is >$100. A cost-effective replacement has not be found.

Thermal grippers: The Hot Hand thermal grippers are available from VWR for $22.37 each (catalog #56614-528).

Micropipettors: The FisherBrand micropipettors are from Fisher Scientific. The 5–40 µl size is no longer available. The replacements will be a 5–50 µl (catalog #21-377-818) for $262.50. Very similar pipettors are available from Bio-Rad for $250.00, from Carolina for $199.00, and from Edvotek at $179.00.

Yellow pipette tips: The yellow tips are from Fisher and are available in bulk bags of 1000 tips for $16.25 (catalog #02-681-2). Bags of 1000 tips are also available from Carolina ($46.50), Edvotek ($40.00), and Bio-Rad ($28.80).

Fixed volume pipettors: The fixed volume micropipettes are $24.00 each from Bio-Rad (10-µl: 166-0512EDU; 20-µl: 166-0513EDU). The same (or similar) pipettors are also sold by Carolina Biological ($27.00 each) and Edvotek ($22.00 each). (Edvotek sells 11 sizes, from 5 µl to 200 µl.)

Practice pipetting stations: Practice pipetting gels are being made at Fralin. If you would like specific directions on how to prepare your own, just email me.

1 kb Plus DNA Ladder: The 1 kb Plus DNA is catalog #10787026 from Invitrogen, $479.00 per mg (plus shipping of ~$60.00). After this is diluted to the working concentration of 0.1 µg/µl, it is good for 1000 applications. The ladder is also available in a 250-µl size for $154.00 plus shipping (catalog #10787018).

Plasmid DNA: The plasmid DNA is prepared at Virginia Tech, but a number of companies have plasmid DNA available for use in classroom restriction digests and mapping.

Chemicals: The chemicals used in the kit are available from a variety of sources, including companies that specialize in supplying schools, such as Carolina Biological Supply Company, Bio-Rad, and Edvotek. A number of companies offer the buffers pre-made. I've just listed the cost for the chemicals from two sources, Sigma Chemical Company and Carolina.

chemical	unit	Sigma	Carolina
agarose	25 g	$59.70	$56.50
EDTA	100 g	22.90	11.95
Tris	500 g	76.60	33.95 (250 g)
Orange G dye	25 g	38.00	21.25
methylene blue	25 g	61.40	14.95
glycerol	500 ml	54.50	10.50

Companies:

Bio-Rad Life Science Education
https://www.bio-rad.com/en-us/education

Carolina Biological Supply Company
http://www.carolina.com

Edvotek
http://www.edvotek.com

Fisher Science Education
Fisher webpage

FOTODYNE Incorporated
http://www.fotodyne.com/

GE Healthcare Life Sciences
http://www.gelifesciences.com/webapp/wcs/stores/servlet/Home/en/GELifeSciences-US/

Invitrogen Corporation
https://www.invitrogen.com/site/us/en/home.html

Modern Biology Inc
http://www.modernbio.com/

Promega
Promega USA website]

Sigma Chemical Company
http://www.sigmaaldrich.com/united-states.html

SOLUTIONS

6x Orange G tracking dye: To prepare 100 ml of 6X tracking dye, mix 0.5 g orange G dye, 5 ml of 50x TAE buffer, 45 ml glycerol, and 50 ml water.

50X TAE buffer: To prepare 1 liter, add 242 g Tris and 100 ml 0.5 M EDTA, pH 8, to 500 ml distilled water. Mix, then add 57.1 ml of glacial acetic acid. Mix, and bring to final volume of 1 liter. The pH (unadjusted) is ~8.2–8.3. Store at room temperature.

The 1x TAE concentration (used for preparing gels) is 40 mM Tris, 20 mM acetic acid, and 1 mM EDTA. The 0.25x TAE concentration (used for running buffer) is 10 mM Tris, 5 mM acetic acid, and 0.25 mM EDTA.

Practice pipetting dye is 20% glycerol in water with 1 mg/ml bromphenol blue.

50x methylene blue: The 1x working concentration of methylene blue is 0.025%, which means it is 0.025 g of methylene blue in 100 ml of water. A 50x solution is 1.25%, so it is prepared by adding 1.25 grams of methylene blue to 100 ml of water**.**

The working concentration (~0.1 µg/µl) of the **1 kb Plus ladder** is prepared by mixing:

125 µl DNA ladder
487 µl 6x bromphenol blue tracking dye (30% glycerol, 0.25% bromphenol blue)
375 µl 10 mM Tris, pH 8
13 µl 5 M NaCl

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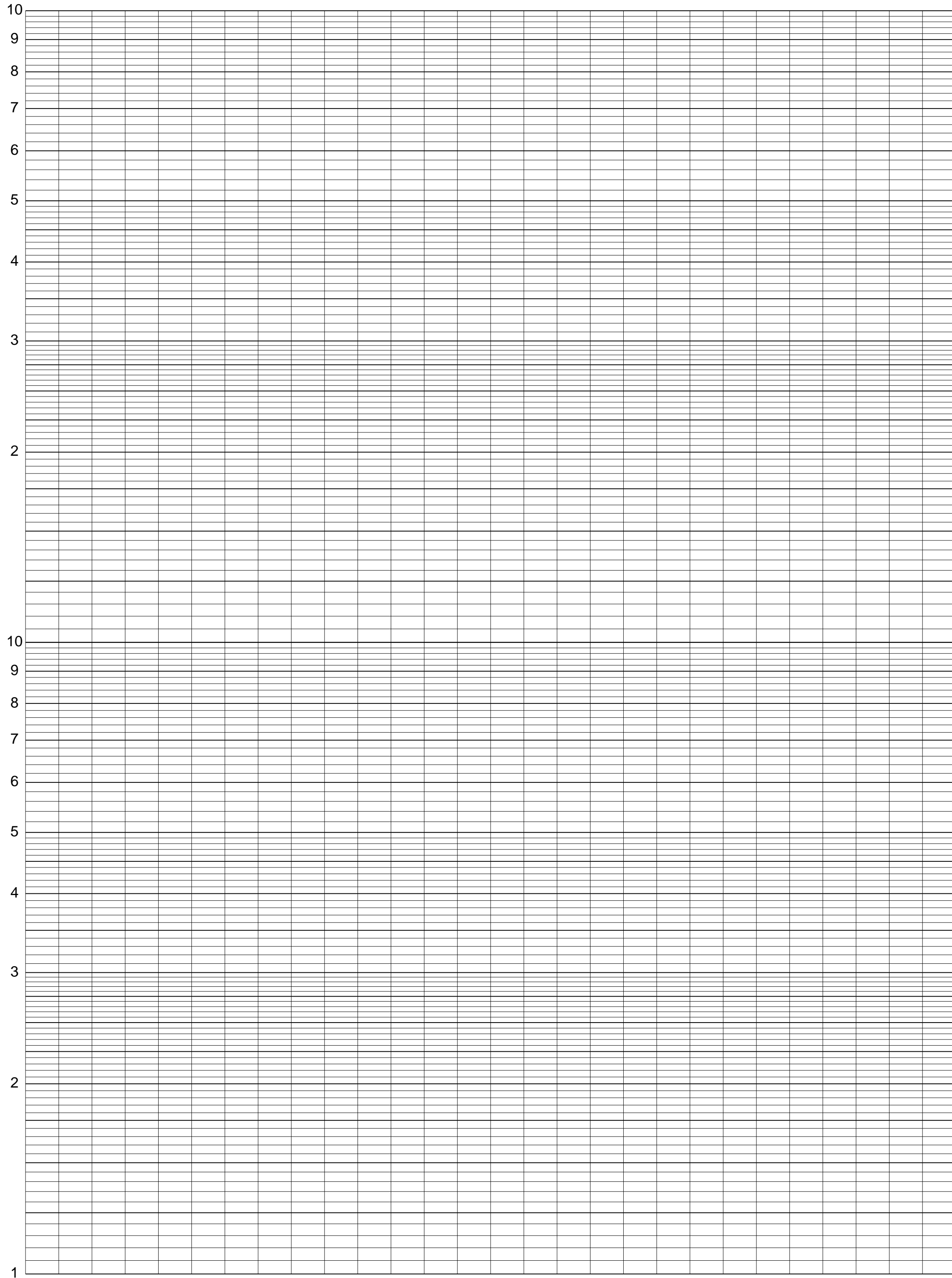
DIRECTIONS FOR PACKING & RETURN SHIPPING

Please return the checklist with the kit.

- Please repack the equipment carefully. Place bubble sheets on the bottom of the box and place fragile items in bubble bags. Please use excess packing material in any empty space at the top and sides of the trunks. **Please do not use Styrofoam peanuts!**
- When you place the **power supplies** in the trunks, please place them **flat and right side up** in their bubble wrap bags. If they are in any other position, there is stress placed on the internal mechanisms, and they work themselves loose from the bases.
- Please be sure to return the **power cords** for the Bio-Rad power supplies with the kit.
- When you repack the **Bio-Rad electrophoresis chambers**, please ship the casting trays and casting gates in the gel boxes, as they were when you received them. Pack the combs in a bubble bag.
- Please return any Nalgene bottles that were included with your shipment. We can re-use these. Any items that are not shaded on the checklist are expected to be returned.
- Please make a note of any damaged equipment and ***please*** do not attempt repairs. Place a note on any problem equipment so we'll know which piece isn't working right.
- Seal the trunks with the cable ties provided. Please make sure that the cable ties are secure. Please note that there is a right way and a wrong way to insert the tab in the cable tie. Look at the end you put the tab through. The tab should be put in from the side that is smooth with the tie, not the end that sticks out. If it is done the wrong way, the cable tie will open when you pull on it. Please test the cable tie by pulling to be sure that you've done it correctly.
- Call Federal Express for pickup. The FedEx phone numbers are 1-800-238-5355 or 1-800-GO-FEDEX. They require at least **three** hours notice before pickup, but you can call as soon as you know when the trunks will be available for pickup. Please be certain to return the trunks on or before the day that was scheduled. We frequently have only a day for turnaround before the trunks are sent out again.
- Remove any old FedEx labels from the trunks. Place the prepared FedEx return labels, ready to go in plastic sleeves, on both trunks.



Free Logarithmic Graph Paper from http://incompetech.com/graphpaper/logarithmic/