Sequencing a Genome:

Inside the Washington University Genome Sequencing Center

Activity Supplement

Paper Terminators

(DNA Sequencing)

Project Outline

The multimedia project *Sequencing a Genome: Inside the Washington University Genome Sequencing Center* is aimed at increasing the scientific literacy of biology students in the technology of genomic sequencing.

The following four video pieces are included on VHS cassette or CD:

- A guided tour of the Washington University Genome Sequencing Center, providing a look at the labs and offices that make up the preparation, production, and data management facilities. Includes animated explanations of the processes used to sequence genomic DNA.
- Exploration of current genomic research in pathogenic bacteria through an interview with a molecular microbiologist.
- Information about careers available at the Genome Sequencing Center presented through interviews with actual employees.
- An animated explanation of the chemistry of cycle sequencing using dideoxynucleotides.

Additional CD features include scripts of the video pieces, links to additional resources, and a glossary of terms.

As the scientific procedures presented in the video tour are complex, simple activities were specifically designed to better explain and reinforce the key concepts of restriction fragment mapping, PCR, sequencing, and electropherogram interpretation. The following paper modeling activity is an inexpensive and simple solution to presenting DNA sequencing.

Acknowledgments
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PAPER TERMINATORS
TEACHER MANUAL

Lesson Overview

Paper Terminators is designed to provide your students with a deeper understanding of PCR-based DNA sequencing through reading, hands-on paper modeling, and completion of a worksheet. In this unit, the students will read about the components of dye terminator sequencing reactions, how the reactions work, and how the products of the reactions are sorted by size in capillary electrophoresis. They will then integrate these new ideas in a paper modeling activity. In Part I, the students will work in pairs to synthesize 12 partial copies of a DNA template in 12 cycles of PCR. In Part II, they will sort their DNA fragments by size and determine the nucleotide sequence of the original DNA template. The worksheet will ask them to define and explain terms and concepts covered in the reading and modeled in the activity, and make suggestions as to possible uses of DNA sequencing technology.

Paper Terminators is specifically designed to follow Paper PCR, as a fundamental understanding of PCR is critical to understanding PCR-based dye terminator sequencing.

Timeline

The background reading, paper modeling, and worksheet require 50 minutes to complete.

Materials

For each pair of students:

- 1 DNA Template (blue)
- 16 Sequencing Primers (green)
- 1 Sequencing Grab Bag (envelope) containing the following:
  - 18 As (white)
  - 30 Ts (white)
  - 30 Cs (white)
  - 30 Gs (white)
  - 12 Terminator As (red)
  - 12 Terminator Ts (red)
  - 12 Terminator Cs (red)
  - 12 Terminator Gs (red)
- 1 roll of transparent tape

Advance Preparation

- You will need to print and cut out one complete set of copy masters plus an additional copy of the Sequencing Primers page per pair of students. The paper pieces will be much more durable if they are laminated before they are cut out and lamination will ensure that you will be able to use the models year after year.
- Black print on white paper may be used, but using different colored papers will help students recognize the difference between the single-stranded DNA Template, Sequencing Primer, Nucleotides, and Terminator Nucleotides. If you choose to do

this, we suggest you print the DNA Template page on blue paper, the Sequencing Primers page on green, the Nucleotides pages on white, and the Terminator Nucleotides pages on red.

- Cut out the single-stranded DNA Template and tape it together as indicated. It should result in a 16 base long fragment with the following nucleotide sequence:

  AGTCACGCAGTTCTAG

Hints and Troubleshooting

- You may find it necessary to go over the background information and demonstrate assembly of one DNA fragment to the class before allowing the student pairs to work on their own. We suggest making overhead transparencies of the copy masters.
- If the DNA Template appears to be too long for your students to handle, the last segment can be eliminated, resulting in the following 11 base fragment:

  AGTCACGCAGT
- Because there is only a single DNA Template piece to be used over and over again, it will help your students if they tape the DNA Template to a solid surface, setting aside each finished DNA fragment as it is generated. The Student Manual suggests taping to the desktop. If you do not want your students taping to their desktops you will need to provide an alternative surface.

Answers to Student Worksheet

1. Record the Terminator Nucleotide sequence determined by you and your partner during Part II of the modeling activity.

   TGCGTCAAGATC (There should be spaces indicated for any missing nucleotides.)
2. Is the sequence above identical to the DNA Template in the activity? _____*No*_____ Why or why not?

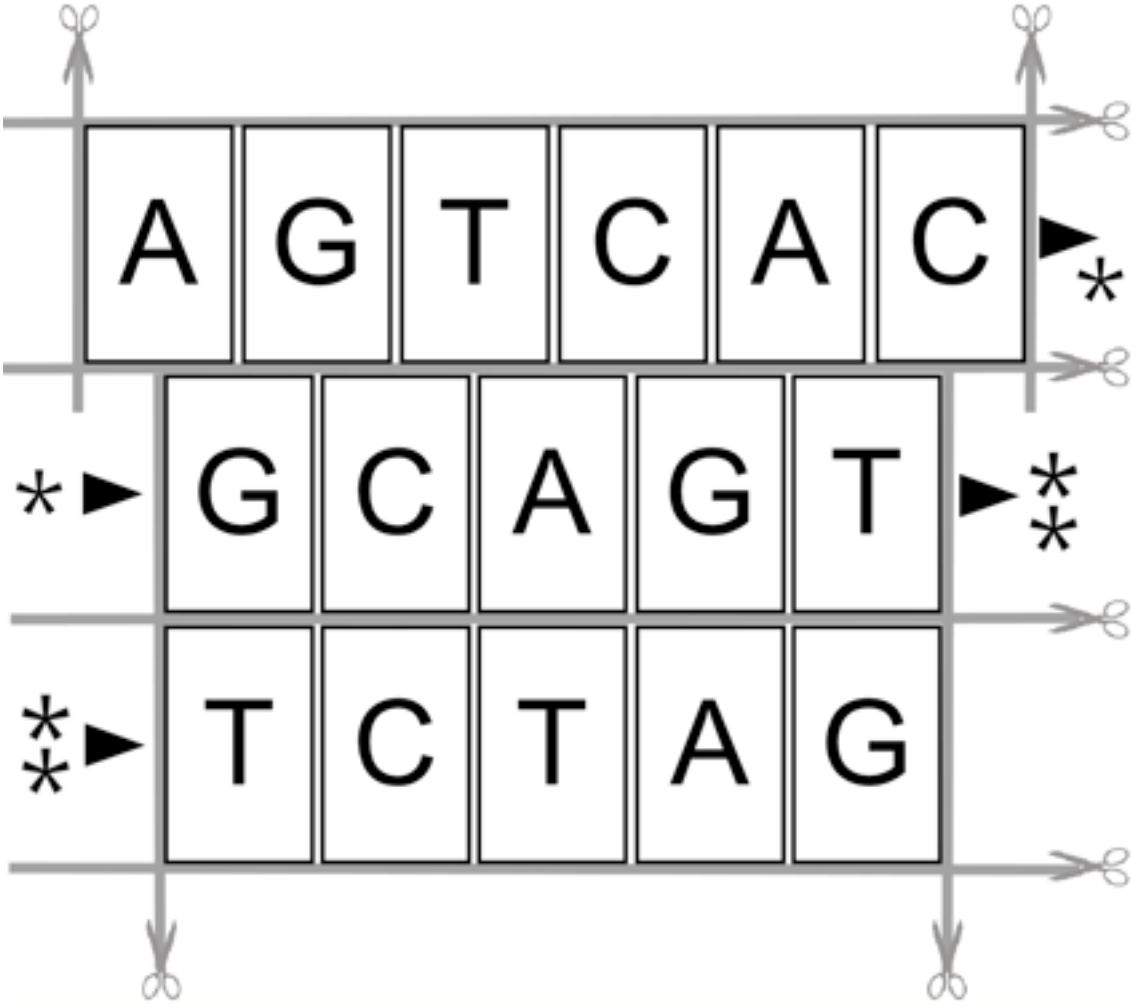
   It cannot be identical to the DNA Template because DNA synthesis creates a new DNA strand that is complementary to the DNA Template. All of the DNA fragments synthesized in the sequencing reaction were complementary to the DNA Template.
3. If there were any empty places in your sequence, or positions for which no nucleotide was determined, what could you do to solve this problem?

   Perform additional cycles of PCR until all possible DNA fragments are synthesized.
4. What are the five main components of a PCR-based dye terminator reaction?

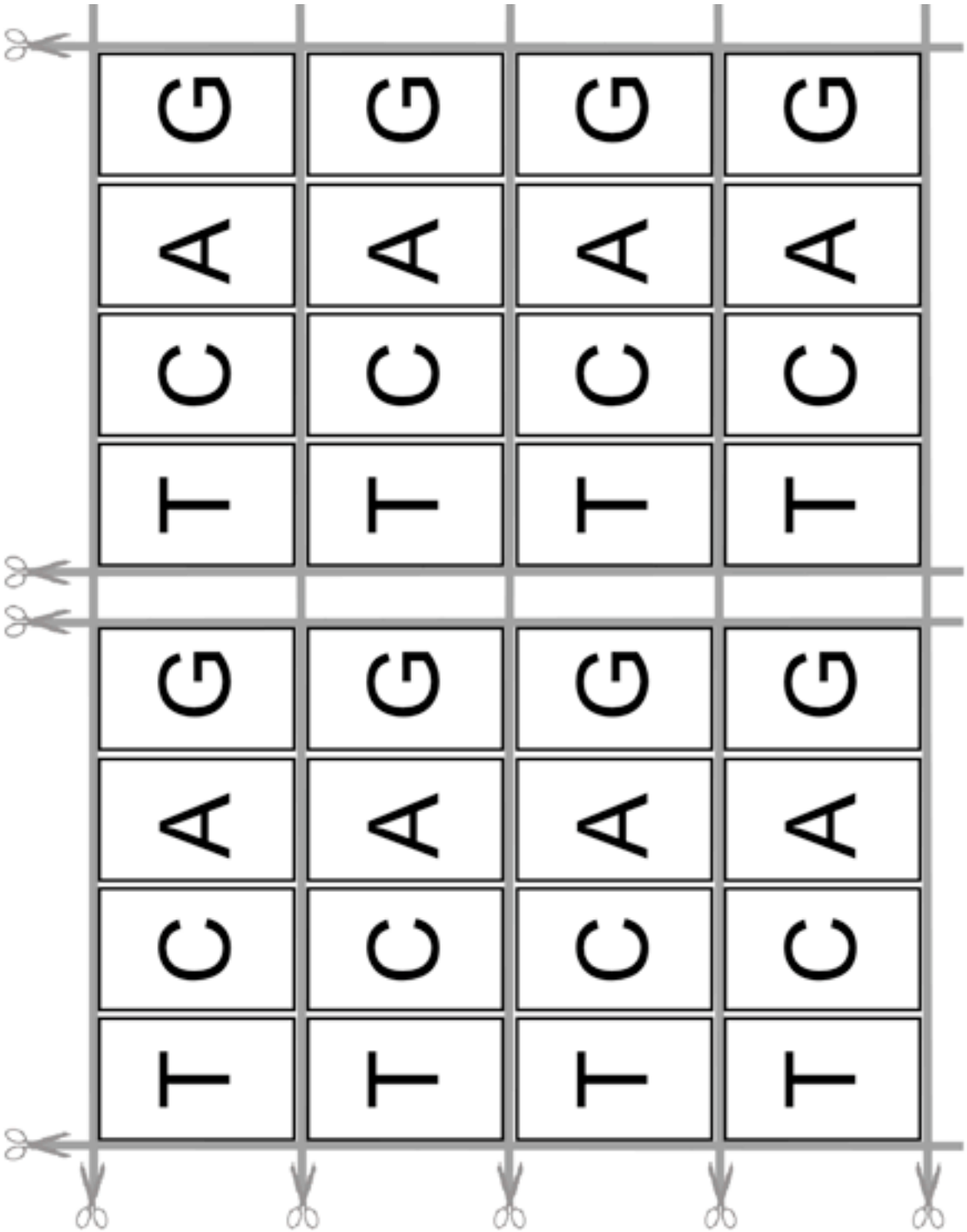
   DNA template, Taq DNA polymerase, oligonucleotide primers, nucleotides, and terminator nucleotides.
5. ___*Terminator nucleotides*_____ are designed to stop DNA synthesis.
6. Changes in ____*temperature*__ are the key to making the dye terminator reaction work.
7. How is the way you and your partner determined the terminating base on a DNA fragment during the activity different from the way a sequencing machine would "read" that same terminating base?

   We were able to actually read the nucleotide base printed on the paper. A sequencing machine can only capture an image of the fluorescent color emitted by the DNA fragment when a laser beam hits it as it exits the capillary tube.

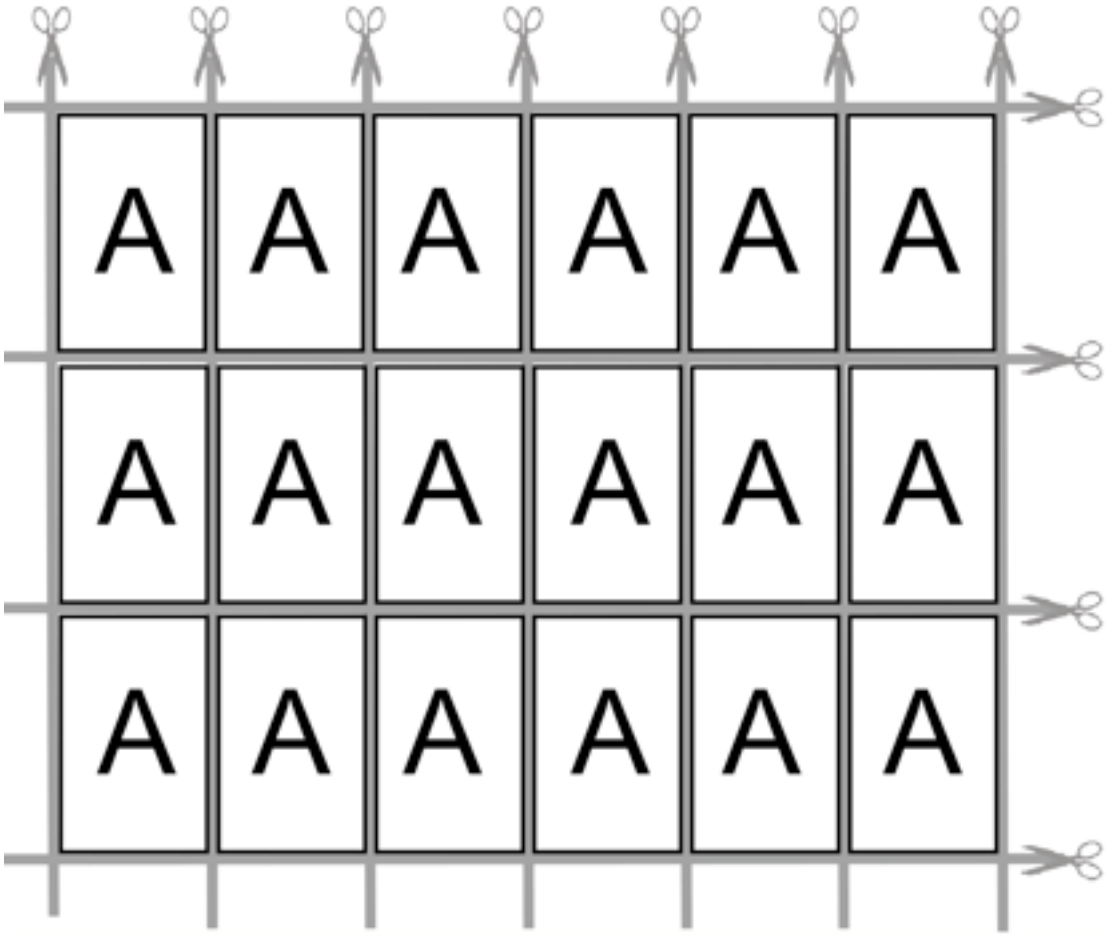
COPY MASTER
DNA Template (blue paper)



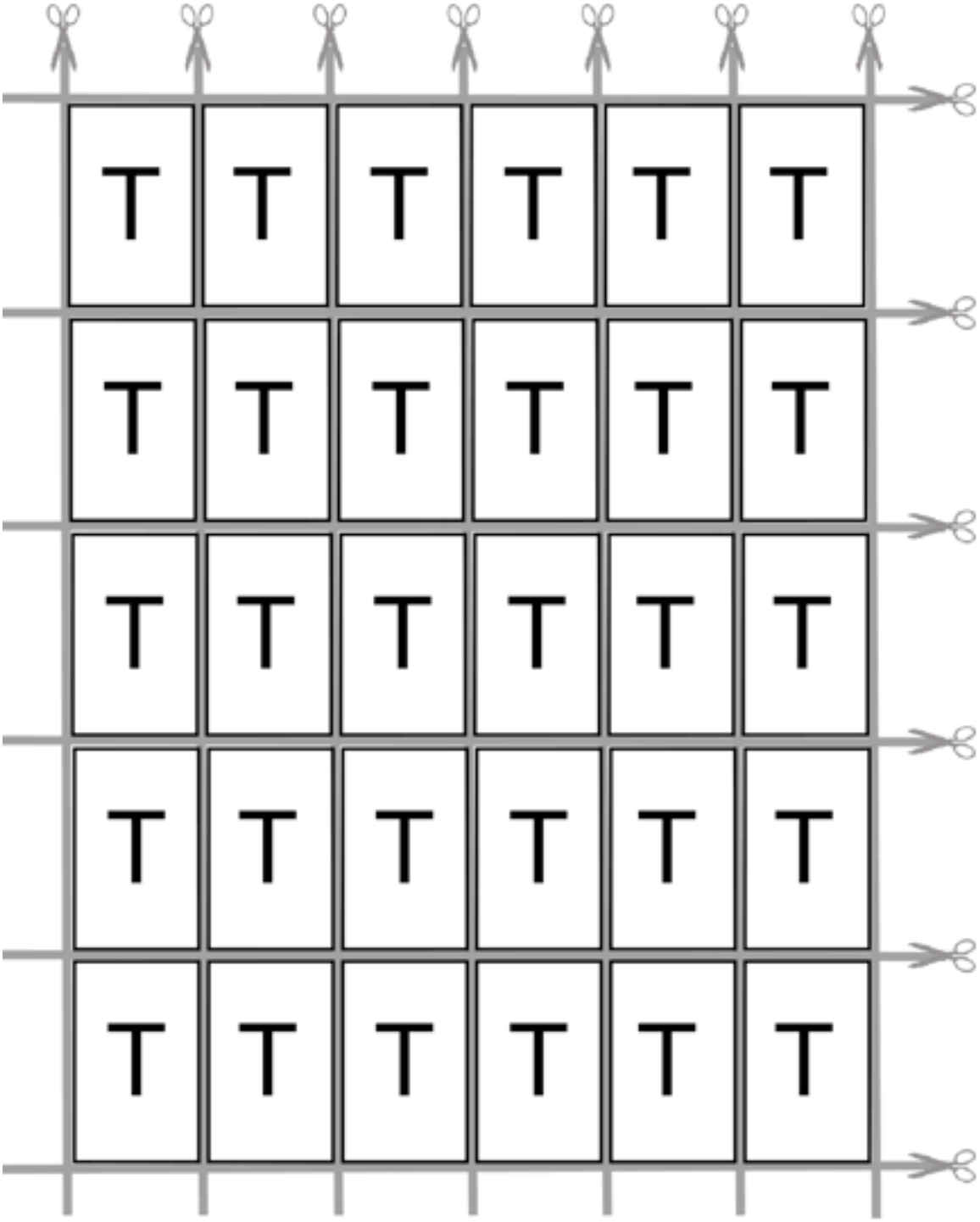
COPY MASTER
Sequencing Primers (two copies of this page are necessary for a complete model set) (green paper)



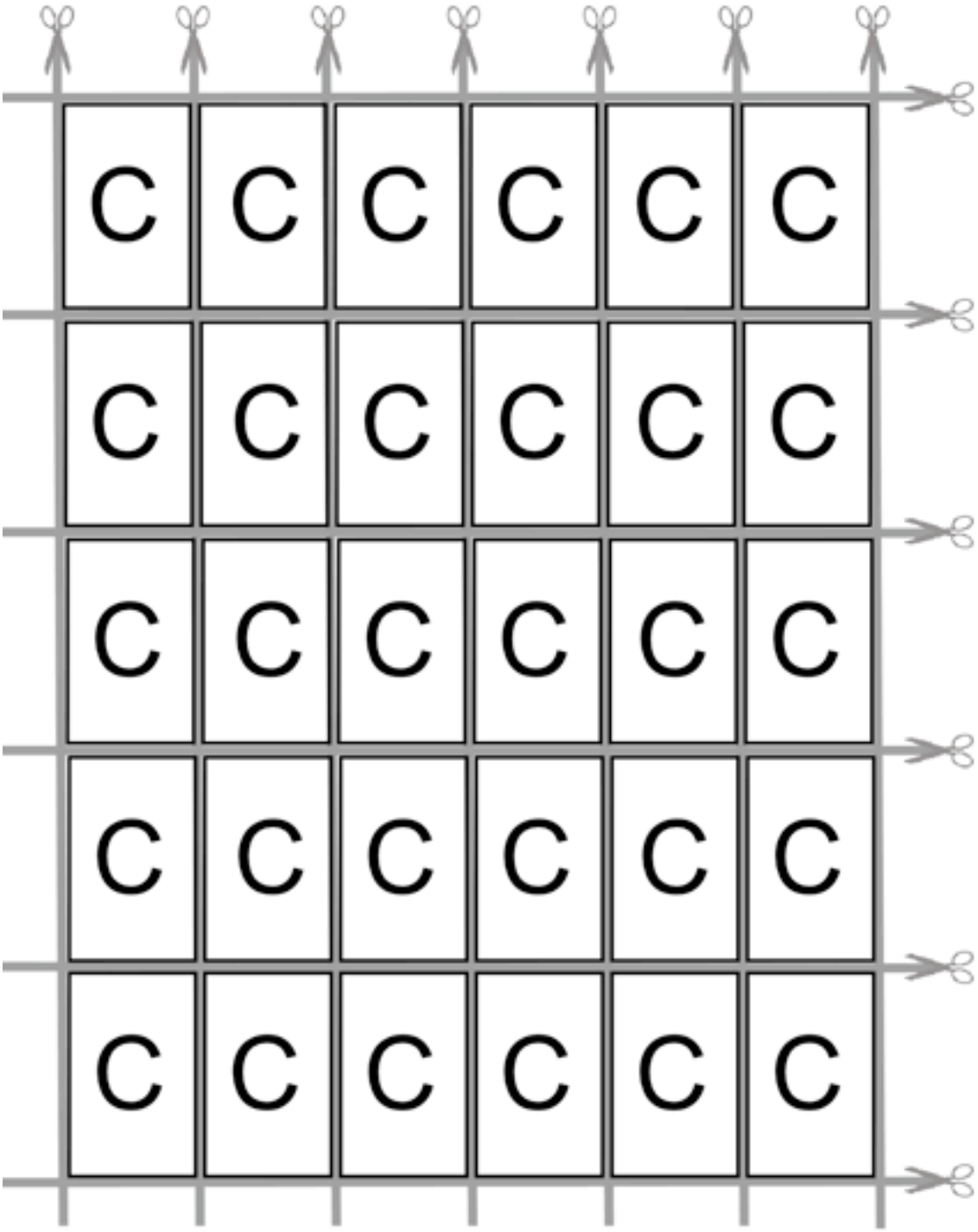
COPY MASTER
Nucleotide A (white paper)



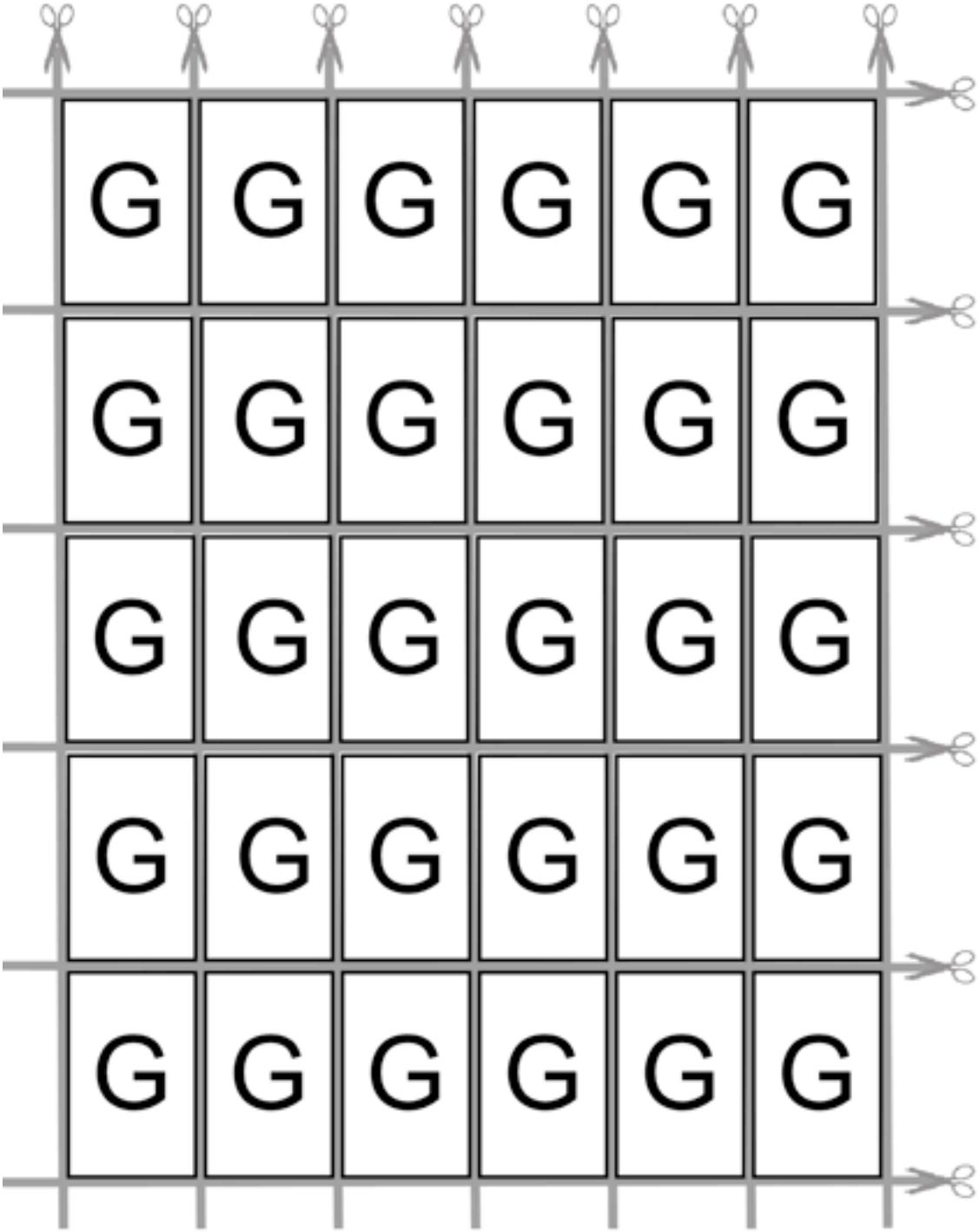
COPY MASTER
Nucleotide T (white paper)



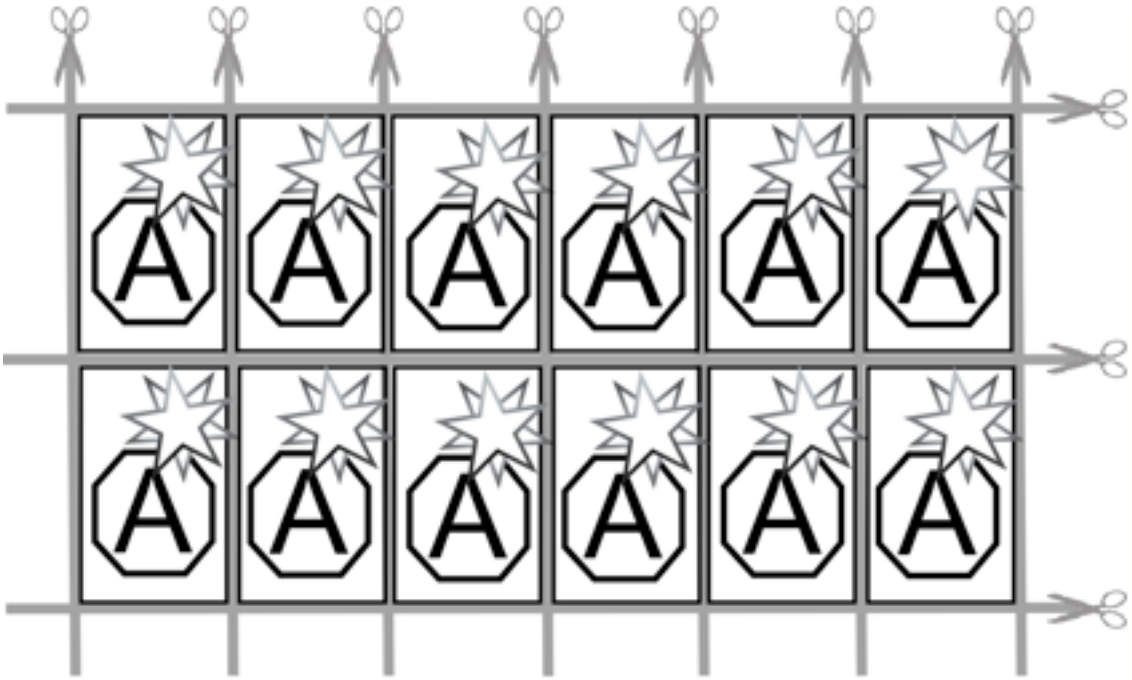
COPY MASTER
Nucleotide C (white paper)

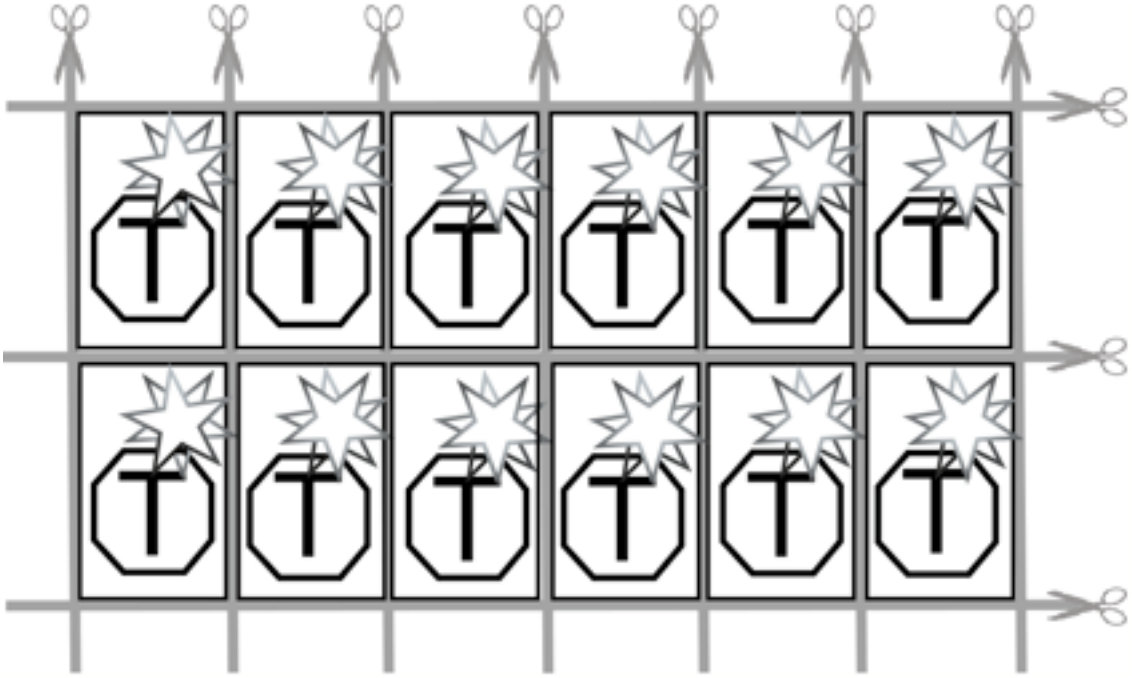


COPY MASTER
Nucleotide G (white paper)

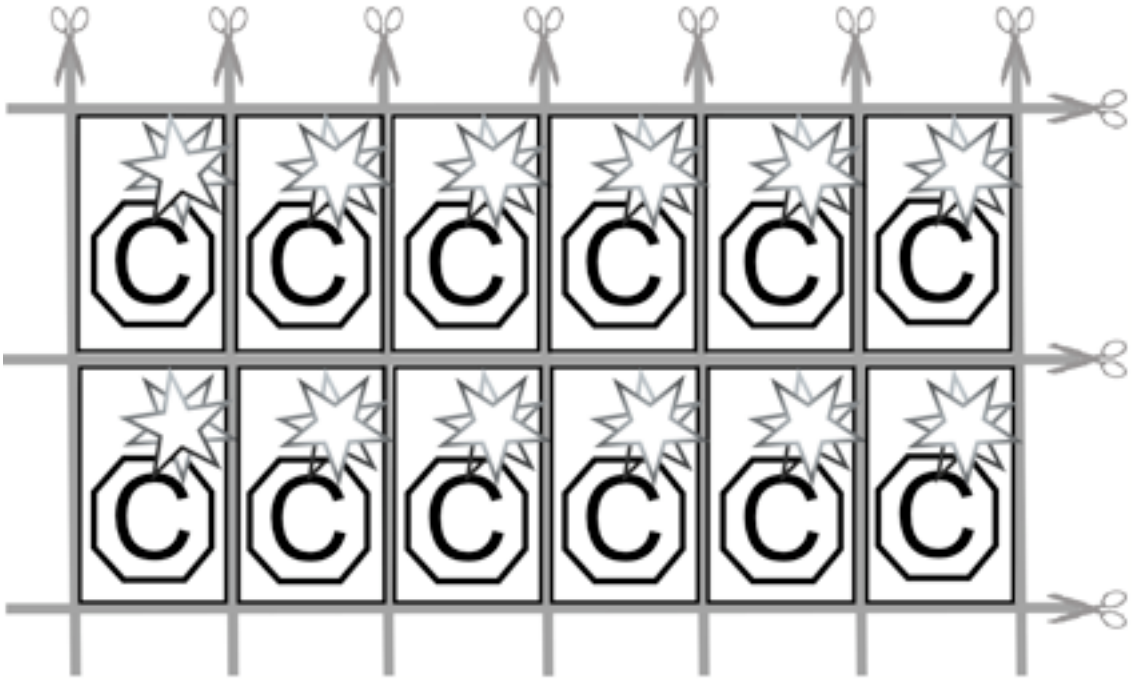


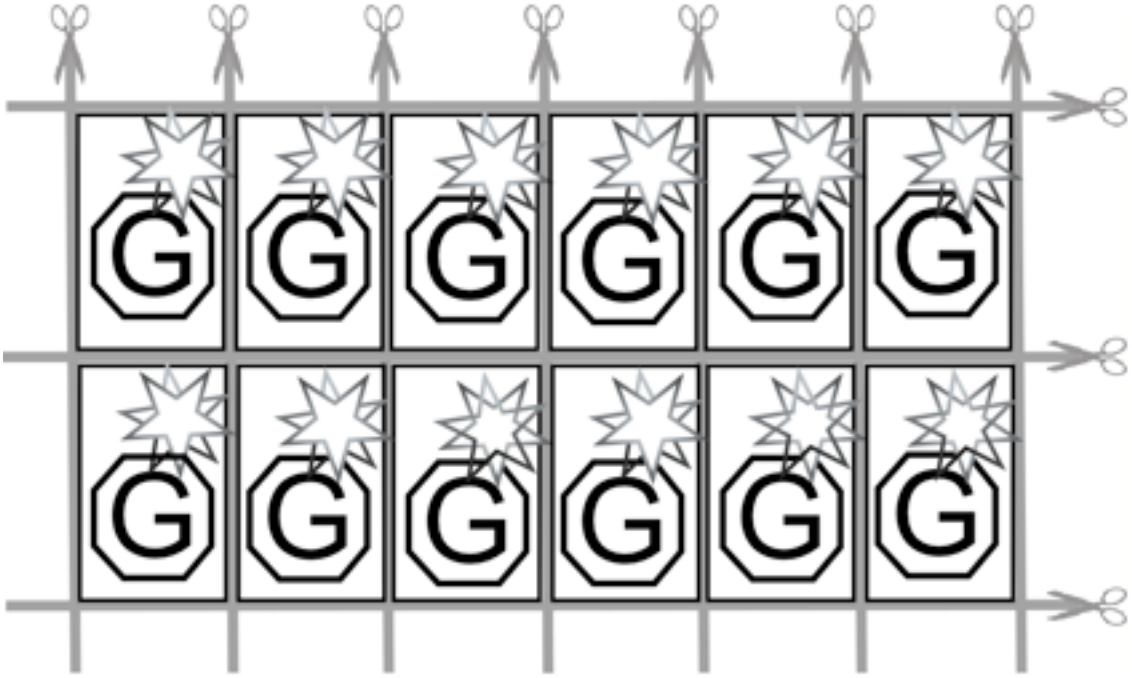
COPY MASTER
Terminator Nucleotides A and T (red paper)





COPY MASTER
Terminator Nucleotides C and G (red paper)





PAPER TERMINATORS
STUDENT MANUAL

Background Information

You have most likely heard of the Human Genome Project and its publication of a first draft of the human genome sequence in February 2001. But, you may be wondering exactly what it means to "sequence DNA."

Sequencing DNA is the process of determining the exact order of the nucleotide bases (A, T, C, G) that make up a particular stretch of DNA. If that stretch of DNA is a gene, then a researcher uses certain procedures to figure out the exact order of bases that make up that gene. Why would anyone want to do this? Knowing the sequence of a gene's DNA can be very important because it allows a researcher to identify special features, like mutations, which may affect that gene's function.

Scientists are also interested in the idea of sequencing DNA on a significantly larger scale. **Genomic sequencing** is determining the exact order of nucleotide bases in every bit of DNA in an organism. As you can guess, in the case of the Human Genome Project, researchers are determining the exact order of nucleotide bases in all of our 23 chromosomes, and even in our mitochondrial DNA. Data from this project will allow us to better understand how our bodies work, and how they sometimes don't. Scientists and doctors will be able to more effectively study and treat certain diseases, genetic disorders, and even cancers, because of data from the Human Genome Project.

The modern DNA sequencing technology used in genome sequencing projects is a fusion of three significant biotechnological breakthroughs – (1) the chain termination method of sequencing developed by Fred Sanger in 1977, (2) the PCR technique developed by Kary Mullis in 1985, and (3) the dideoxynucleotide fluorescent dye chemistry developed by Lee Hood and Lloyd Smith in 1986.

The Sanger chain termination method (sometimes called "Sanger sequencing") is a way to create identical sequences of DNA that differ only in length by a single nucleotide.

ATCGATCGATCG
ATCGATCGATC
ATCGATCGAT
ATCGATCGA
ATCGATCG
ATCGATC

It is based on two facts of DNA synthesis. First, when the four deoxynucleotide bases (A, T, C, G) are put in the presence of a DNA polymerase, synthesis of a new strand of DNA will begin when a short single-stranded DNA primer is bound to a single-stranded DNA template. (This should sound familiar to you – think PCR!) And second, if dideoxynucleotide bases, or terminator nucleotides, are included in the reaction along with regular deoxynucleotide bases, DNA elongation will stop when a dideoxynucleotide base is incorporated. The molecular chemistry of a terminator nucleotide does not allow any additional nucleotides to be added.

The random assortment of free-floating nucleotides and terminator nucleotides in the Sanger sequencing reaction ensure that the newly synthesized DNA fragments are each terminated at a different place along the DNA template sequence. Each time the reaction mixture is heated for denaturing, cooled for annealing, and warmed for extension, more DNA fragments are created. The goal is for the reaction to result in an array of DNA fragments that have only a single nucleotide difference in length. So, when we put the Sanger method together with the multiple rounds of temperature cycling of PCR technology, we have a way to generate an array of DNA fragments of differing lengths, all complementary to the same DNA template sequence.

DNA template	TAGCTAGCTAGC
array of DNA fragments	ATCG*
	ATCGAT*
	ATCGATCGATCG*
	ATCGATCGATCG*
	ATCGATCG*
	ATCGATC*
	ATCGATCGATC*
	ATCGATCGAT*
	ATCGATCGAT*
	ATCGATCGA*
	ATCGATCG*
	ATCGATC*
	ATCGATCG*

Now let's put this together with a way to tell the terminator nucleotides apart. Labeling each of the terminator nucleotides with a unique fluorescent dye allows for the identification of the terminating base on any DNA fragment generated by the Sanger method described above. The fluorescently labeled terminator nucleotides each have their own specific color. A is green, T is red, G is yellow, and C is blue. After the multiple rounds of temperature cycling, any DNA fragments that can fluoresce green must have an A at the terminating nucleotide. Those that can fluoresce red must have a T at the end. Those that can fluoresce yellow must have a G at the end and those that can fluoresce blue must have a C at the end of the terminating nucleotide.

In summary, a **dye terminator reaction** is a modified Sanger sequencing reaction that uses PCR technology and fluorescently labeled terminator nucleotides to synthesize an array of DNA fragments of differing lengths, all complementary to the same DNA template sequence.

Okay, this sounds great, but a dye terminator reaction doesn't tell you the sequence of the DNA template. One more technique is needed for that – gel electrophoresis.

All of the fluorescently labeled DNA fragments created in the dye terminator reaction need to be separated by size. This is possible with **gel electrophoresis**. The electrophoresis system used in genomic sequencing projects is a part of a sequencing machine and is made

up of capillaries, or small tubes, containing polyacrylamide gel. Dye terminator reactions are loaded into the capillaries, one reaction per tube. The DNA fragments from each dye terminator reaction are put in order from smallest to largest as they migrate through the capillaries. As a DNA fragment exits a capillary tube, it is hit with a laser beam that excites the fluorescent dye attached to its terminator nucleotide. A camera captures an image of the fluorescence, a computer converts it to a readable form called an electropherogram, and the corresponding terminating nucleotide is identified! The data in the electropherogram is complementary to the sequence of the DNA template.

unknown DNA template	? ? ? ? ? ? ? ? ? ? ? ?
dye terminator fragments	? ? ? ? ? ? ? ? ? ? ? ? G* ? ? ? ? ? ? ? ? ? ? ? C* ? ? ? ? ? ? ? ? ? ? T* ? ? ? ? ? ? ? ? ? A* ? ? ? ? ? ? ? ? G* ? ? ? ? ? ? ? C* ? ? ? ? ? T* ? ? ? ? A* ? ? ? G* ? ? C* ? T* A*
electropherogram data	ATCGATCGACTG
DNA template sequence	TAGCTAGCTAGC

So, to bring this all together, you can put an unknown piece of DNA template into a dye terminator reaction, temperature cycle the reaction in a PCR machine, load the DNA fragment products of the reaction into a sequencing machine, the fragments will be ordered from smallest to largest by electrophoresis, a computer will "read" the last base on each DNA fragment, and the sequence of the original unknown DNA template can be determined. Do not worry if this is not clear to you yet. You will have a better understanding after completing the Paper Terminators activity.

Let's review how the products of the dye terminator sequencing reaction are generated. You need the following five components in the reaction:

DNA Template – This is double-stranded genomic DNA isolated from the cells of the organism being studied. It can be human DNA, plant DNA, mouse DNA, bacterial DNA, whatever DNA you would like to sequence! The point is that if you would like to sequence something using the dye terminator reaction method, then you must have a master template from which to start.

***Taq* DNA Polymerase** – This enzyme can add complementary nucleotides to a DNA strand during DNA synthesis. It is similar to the human DNA polymerase responsible for copying your genome every time one of your body cells divides.

Sequencing Primers – These are short pieces of single-stranded DNA that match up to DNA sequences of genomic DNA that you would like to sequence. When they have bound to the complementary bases on the genomic DNA template strand, they show the *Taq* where to start DNA synthesis.

Nucleotides – Free floating single deoxynucleotides must be present in the reaction because they are what the *Taq* puts in place during DNA synthesis.

Terminator Nucleotides – Free floating dideoxynucleotides linked to fluorescent dyes are the critical component. They are designed to stop DNA synthesis by blocking the ability of *Taq* to put any additional nucleotides in place.

As in PCR-based DNA amplification reactions, the dye terminator reactions occur in a **thermocycler**. And likewise, changes in temperature are the key to making the dye terminator reaction work. Let's review the temperature cycle.

STEP 1 – DENATURING

The reaction mixture is heated up to 96˚C so that the double-stranded DNA template denatures and becomes single-stranded. (This high temperature breaks the hydrogen bonds between the complementary bases in double-stranded DNA.)

STEP 2 – ANNEALING

The reaction mixture is cooled down to 50˚C so that the sequencing primer can base pair with, or anneal to, the DNA template. (This cooler temperature allows hydrogen bonds to form between complementary bases.)

STEP 3 – EXTENSION

The reaction mixture is warmed up to 60˚C, so *Taq* DNA polymerase can perform DNA synthesis. *Taq* can recognize a sequencing primer as a starting point for DNA synthesis. It is able to put free-floating nucleotides into the correct places along the DNA template so that a new complementary strand of DNA is extended from the primer. However, when a fluorescently labeled terminator nucleotide is put in place, *Taq* is no longer able to add more nucleotides and DNA synthesis is stopped.

If this all seems very complicated that is because it is! However, you can understand it and the following activity will help you see exactly what is going on in dye terminator reactions and DNA sequencing.

<u>Activity Overview</u>

In Part I of this activity, you and a partner will play the part of *Taq* DNA polymerase as you randomly synthesize several fragments complementary to a particular DNA sequence of interest. Your whole class will represent several dye terminator reactions occurring simultaneously in a single reaction tube. Your teacher will play the part of the PCR machine, directing the timed changes in temperatures during 12 cycles of PCR.

In Part II of this activity, you and your partner will play the part of a sequencing machine. You will work together to separate and sort your newly synthesized DNA fragments as if you have loaded and run your sequencing reaction products in a capillary gel

electrophoresis system, and you will "read" the sequence of the terminating nucleotides as each fragment exits the capillary.

Materials

For each pair of students:

- 1 DNA Template (blue)
- 16 Sequencing Primers (green)
- 1 Sequencing Grab Bag (envelope) containing the following:
  - 18 As (white)
  - 30 Ts (white)
  - 30 Cs (white)
  - 30 Gs (white)
  - 12 Terminator As (red)
  - 12 Terminator Ts (red)
  - 12 Terminator Cs (red)
  - 12 Terminator Gs (red)
- 1 roll of transparent tape

Procedure – Part I

To simplify things, we are going to assume that the first step of the first cycle of PCR has already occurred. This means the PCR machine has already reached a temperature of 96˚C and your DNA Template has denatured and become single-stranded.

1. Locate all of the materials listed above. Lay out your single-stranded DNA Template in front of you and your partner and secure it to the desktop with tape. Wait for your teacher (the PCR machine) to start the second step of the first cycle of PCR.

2. Your teacher will indicate that the PCR machine has reached a temperature of 50˚C. You and your partner may now anneal a Sequencing Primer to the complementary bases on your single-stranded DNA Template. Lay your Sequencing Primer down so that the correct bases match up to those on your DNA Template.

3. Your teacher will indicate that the PCR machine has heated up to a temperature of 60˚C. You will now act as *Taq* DNA Polymerase, extending a complementary strand of DNA out from your Sequencing Primer, one base at a time. To do this, you or your partner will blindly choose a nucleotide from the Sequencing Grab Bag.

   a. If your selected nucleotide does NOT correctly match up to the corresponding base on the DNA Template, then you must put it back in the Sequencing Grab Bag and select again.
   b. If your selected nucleotide correctly matches up to the corresponding base on the DNA Template then you may lay it down and tape it to the end of the Primer.

c. If your selected nucleotide correctly matches up to the corresponding base on the DNA Template AND it is a Terminator Nucleotide, then lay it face down and tape it to the end of the Primer. Your new strand is finished.

4. Once you have encountered a Terminator Nucleotide, your new strand of DNA is finished. (Even if it is not as long as the DNA Template!) Wait for the rest of your class to finish extending their new DNA fragments.

5. Your teacher will indicate that the PCR machine has reached a temperature of 96˚C. You may now denature your double-stranded DNA fragment. Slide the newly synthesized DNA fragment over to one side.

6. Follow your teacher's instructions through the temperature changes for 11 more cycles of PCR (Steps 2 through 5). When you have finished, you and your partner should have 12 fragments of single-stranded DNA in front of you. Some of the fragments may be identical in length, some may vary in length, or you may have 12 fragments of all differing lengths.

Procedure – Part II

Now that you have completed your dye terminator sequencing reaction, you need to separate and sort your DNA fragments according to size and "read" the terminator nucleotide on each fragment.

You should remember that DNA fragments can be separated using gel electrophoresis. Smaller molecules will move faster through a gel than larger molecules, so you should be able to sort your DNA fragments from smallest to largest by running them through a capillary gel electrophoresis system.

Let's pretend you are doing just that, and additionally, the capillary gel electrophoresis system is part of an actual sequencing machine that can "read" and record the terminator nucleotide on each fragment as it drops out of the end of the capillary tube.

1. Turn all of your DNA fragments so that the Sequencing Primer and Nucleotides are face down and only the Terminator Nucleotide is face up.

2. Work with your partner to arrange your DNA fragments from smallest to largest, placing the smallest fragment closest to you and the largest fragment farthest from you.

3. Fragments that are identical in size will travel through a gel together, so you may stack those fragments together in your arrangement. (Note that those fragments of identical length should also have identical Terminator Nucleotides! If they do not, then you must have made a mistake during your sequencing reaction in Part I.)

4. Your smallest DNA fragment will be the first to drop off the end of the capillary tube during electrophoresis. Record the Terminator Nucleotide occurring at the end of your smallest DNA fragment in the first empty position of question 1 on the Paper

Terminators Worksheet. (Remember, the sequencing machine can only "read" the Terminating Nucleotide. You must ignore the nucleotides in the Sequencing Primer and any other regular nucleotides. This is why we have put them face down.)

5. If your next larger DNA fragment is just one nucleotide longer, then you may record the Terminator Nucleotide occurring at the end of that fragment in the second empty position. However, if it is more than one nucleotide longer, then you must skip the appropriate number of spaces before recording the Terminator Nucleotide at the end of that fragment.

6. Go through the rest of your DNA fragments in order, recording the Terminator Nucleotides and possibly skipping spaces as explained above.

7. Complete the rest of the questions on the Paper Terminators Worksheet.

8. Take apart all of your model pieces and return them to the Sequencing Grab Bag.

Name _____ Class Hour _____ Date _____

Paper Terminators Worksheet

1. Record the Terminator Nucleotide sequence determined by you and your partner during Part II of the modeling activity.

   ___ ____ ____ ___ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____

2. Is the sequence above identical to the DNA Template in the activity? _____ Why or why not?

3. If there were any empty places in your sequence, or positions for which no nucleotide was determined, what could you do to solve this problem?

4. What are the five main components of a PCR-based dye terminator reaction?

5. _____ are designed to stop DNA synthesis.

6. Changes in _____ are the key to making the dye terminator reaction work.

7. How is the way you and your partner determined the terminating base on a DNA fragment during the activity different from the way a sequencing machine would "read" that same terminating base?