NEO/SCI STUDENT'S GUIDE

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Neo SCI

New ideas for teaching science

DNA MURDER MYSTERY

Objectives

- ***Simulate*** **the DNA fingerprinting procedure**
- ***Construct*** **an autoradiogram**
- ***Interpret*** **an autoradiogram**
- ***Compare*** **and evaluate DNA fingerprint patterns**
- ***Compare*** **the strengths and limitations of DNA profiling**

Background

On the morning of November 22, 1983, the body of a young woman was discovered just outside the grounds of a mental hospital in Narborough, England. Although it was not immediately evident who committed the crime, a very important piece of evidence was left behind. Police extracted a sample of the murderer's DNA from blood samples found at the crime scene. Three years later, the same DNA results were obtained from a blood sample recovered from another crime scene. The DNA samples eventually turned out to be the clues that solved both crimes.

Shortly after the second murder, the local police arrested a man who worked at the mental institution where the first body was found. He confessed to the second murder, but denied any knowledge of the first one. Because the police were fairly certain that both murders had been committed by the same person, they needed to find some way of proving that the suspect had killed the first victim.

About the same time, Alec Jeffreys, a geneticist at the University of Leicester in England, was studying inherited variation within genes and among individuals as well as the evolution of present-day genes. His work involved looking at a genetic peculiarity known as the "intron". Introns are sequences of "nonsense" or "junk" DNA. In other words, they do not code for a specific protein.

Jeffreys noticed that some introns are made up of the same repeating DNA base pair sequences and that the number of repetitions varies from person to person. For example, in one person a particular base pair sequence may repeat ten times, while in another person the same sequence may repeat twenty-five times. These repetitive intron fragments are called "Restriction Fragment Length Polymorphisms" (RFLPs). Although the DNA sequences of genes - fragments that code for a protein - are fairly constant from person to person, introns are not. RFLPs are unique to each person, except in the case of identical twins.

Jeffreys realized that these variable DNA base pair sequences could be separated using gel electrophoresis and matched with complementary radioactive probes to create an individual-specific DNA banding pattern that would distinguish one person from the next. Because every person has a distinct RFLP banding pattern, Jeffreys called this technique "DNA fingerprinting".

Narborough police asked Jeffreys to verify the suspect's confession by comparing his DNA fingerprint to the one obtained from the crime scene. In addition, police asked another suspect to provide a sample of his blood for DNA analysis.

You have been chosen to undertake a very important task and provide the most important piece of evidence that could actually determine which of the suspects is the murderer by recreating the testing that was done by Jeffreys. You will simulate the DNA fingerprinting technique and determine whether the suspect who had confessed to the crime committed the murders or whether it was someone else.

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Today, DNA fingerprinting is routinely used to test samples of blood, hair, saliva and other bodily fluids left at crime scenes. The results are compared to those of a suspect to establish the individual's innocence or guilt. DNA fingerprint results are not considered irrefutable evidence in court. There is a slight chance that two individuals could have the same RFLP banding pattern. When the medical personnel who do the testing appear at a trial, they tell the jury the statistical probability that two matching samples (e.g. the one from the crime scene and the one from the suspect) came from the same person. This figure is based on the number of known RFLPs that exist in a given population. In some cases, a match can be accurate to within 1 in 10 billion people. Since that's twice the current human population, it would be extremely likely that the suspect did commit the crime.

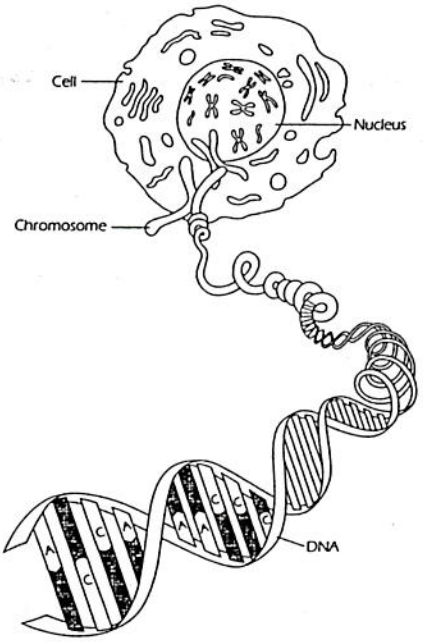
Although DNA fingerprinting is a very accurate technique, some attorneys argue that it can never be accurate enough to prove guilt beyond a reasonable doubt. Many legal challenges have been raised - most recently in the highly publicized case of O.J. Simpson - concerning DNA evidence. However, because the techniques used to produce DNA fingerprints are constantly being improved, the likelihood of mis-identification is much lower today than it was a few years ago.

DNA fingerprinting has many other potential applications. Since half of a person's genome comes from each parent, DNA fingerprinting can be used to determine familial relationships. It can also be used to track hereditary diseases and identify the best matches for organ transplants. This procedure can even be used to ascertain the level of inbreeding in endangered animals like the cheetah and to determine how newly discovered species are related to other organisms on Earth.

What is DNA?

DNA is found in almost all living cells. DNA is an abbreviation for "DeoxyriboNucleic Acid."

It is a chemical structure that forms chromosomes and carries coded information that makes every person an individual. DNA is made up of subunits called "nucleotides" linked together in such a way that the resulting molecule resembles a long, twisted ladder. Each leg of the ladder consists of alternating sugar and phosphate units. The rungs of the ladder are made of pairs of nucleotide bases that are bonded together.



Each nucleotide is made up of the sugar deoxyribose, a phosphate group, and a nitrogen base. While the sugar and phosphate molecules are the same for every nucleotide, the nitrogen bases can be any of the four complementary bases: adenine (A), thymine (T), cytosine (C) and guanine (G). These bases combine in specific ways. Adenine pairs only with thymine, while cytosine pairs only with guanine. The order, or sequence, of nucleotide bases in a molecule of DNA provides instructions used to build amino acids and link them together into proteins. Proteins control most of the chemical reactions that carry out cellular activities. Without protein, our bodies could not grow and function. A piece of DNA that contains all the information needed to build one protein is called a "gene".

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Activity 1

Modeling DNA Fingerprinting

What you need

(Per Group):

24	*Yellow pop beads (Adenine)*
24	*Green pop beads (Thymine)*
44	*Black pop beads (Guanine)*
44	*Blue pop beads (Cytosine)*
136	*Red pop beads (Phosphate)*
27	*Orange pop beads (Radioactive phosphate)*
136	*White-five hole pop beads (sugar group)*
68	*Connectors (hydrogen bonds)*
8	*Cups*
4	*Tag labels*
1	*Restriction enzyme transparency card, Neo/Sci 1*
1	*Restriction enzyme transparency card, Neo/Sci 2*
1	*Autoradiogram transparency card*
1	*Black marker*

What To Do...

Extracting DNA

DNA is first extracted, typically from tissue samples such as blood, skin, hair, semen or saliva. In some cases, due to very small amounts of DNA available for analysis, it is necessary to amplify the DNA by a technique called "Polymerase Chain Reaction" (PCR). DNA from only a few cells or even from a single hair strand can be amplified to generate enough material for fingerprint analysis. DNA extraction is the first step in the analysis and manipulation of DNA, enabling scientists to detect genetic disorders, to produce DNA fingerprints of individuals, and even to create recombinant organisms.

STEP 1

Form an investigative team with eight classmates, divided into four pairs. Each student pair will be responsible for conducting the DNA analysis on one DNA sample. Assign a DNA sample to each student pair and proceed with assembling the DNA samples. Obtain the components needed from your teacher and follow the DNA sequence blueprints to assemble the double DNA strands for each of the following DNA samples:

1. Crime Scene DNA
2. Victim's DNA
3. Confessed Murderer's DNA
4. Suspect 2's DNA

Assemble the nucleotides for the 3' to 5' DNA strand first, then assemble the complementary, 5' to 3' DNA strand. Label each DNA sample with a tag indicating its origin. Repeat this procedure for each DNA sample.

Remember the base pair matching rules:

Guanine always pairs with **Cytosine**

Thymine always pairs with **Adenine**

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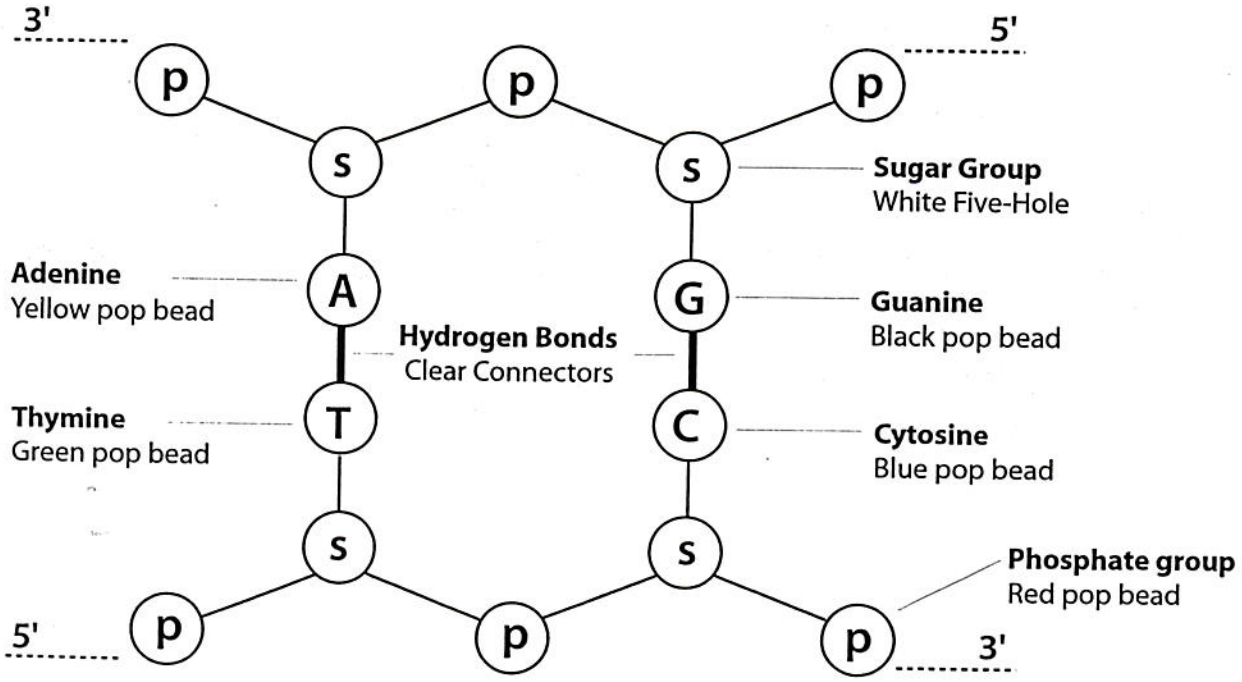
Use the following color scheme:

Adenine	→	Yellow pop beads
Thymine	→	Green pop beads
Guanine	→	Black pop beads
Cytosine	→	Blue pop beads
Phosphate group	→	Red pop beads
Sugar group	→	White pop beads
Hydrogen bonds	→	Clear connectors

Before you proceed with the next step, be sure that the DNA sequence for each sample is correct. Remember, one wrong move and you could be sending an innocent man to prison, or letting the real murderer go free.



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DNA SEQUENCE BLUEPRINTS

Crime Scene DNA

3' 5'

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

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

5' 3'

Victim's DNA

3' 5'

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

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

5' 3'

Confessed Murderer's DNA-Suspect 1

3' 5'

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

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

5' 3'

Suspect 2

3' 5'

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

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

5' 3'

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Using restriction enzymes to cut DNA strands into smaller fragments

The DNA is cut into pieces using special enzymes called "restriction enzymes" that cut DNA at specific base pair sequences. Restriction enzymes, derived from bacteria, recognize specific nucleotide sequences and cut the double-stranded DNA at these sites into fragments. For example, one of the enzymes, **Neo/Sci 2**, that you will use in this investigation, searches for the following sequence in the DNA sample.

3'.....G A A G .....5'

5'.....C T T C .....3'

The enzyme then makes two cuts in the double-stranded DNA as follows:

G A | A G
C T | T C

which creates two fragments:

.....G A
.....C T

and

A G .....
T C .....

STEP 2

Using the two "transparent" restriction enzyme cards provided, cut each strand at their recognition sites. The enzyme **Neo/Sci 1** recognizes the sequence **GG|CC** while **Neo/Sci 2** recognizes the sequence **GA|AG**. Each "transparent" restriction enzyme is passed over each double-stranded DNA until a match is found (i.e. the recognition restriction site of the enzyme matches with the DNA sequence of the DNA sample.)Repeat the process with the second enzyme for each DNA sample. Place the fragments generated from each DNA sample in a separate pile or in labeled cups. Be sure to keep the DNA fragments generated from each DNA sample separately and properly labeled with the identity of each sample.

Determine the number of fragments generated and the length in base pairs of each DNA fragment generated from each DNA sample. Record your results in Table 1.

Table 1

DNA Sample	Number of Restriction Sites	Number of Fragments	Size of each fragment in base pairs
Crime Scene DNA			
Victim's DNA			
Suspect 1(confessed)			
Suspect 2			

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Sorting the DNA using gel electrophoresis

Individual genes or DNA molecules are much too small to be seen directly. However, it is relatively easy to visualize proteins or short segments of DNA and RNA using a technique called "gel electrophoresis". The technique involves the separation and movement of molecules through a gelatin-like material that has been exposed to an electrical field.

An electrophoresis apparatus has five major components - the driving force (electrical current), the sample (DNA, RNA or protein), the support medium (agarose gel), the buffer (liquid) conduction medium) and the detecting system (stain).

Samples, such as DNA fragments, are typically placed in the wells at one end of the gel slab. The gel is then placed in a conductive buffer solution. Electric current is applied at both ends of the electrophoresis chamber, creating an electric field within the gel. Because DNA is negatively charged, DNA fragments always migrate toward the positive pole of the chamber - the end opposite wells. Smaller molecules move more quickly and farther than larger molecules. The different-sized pieces of DNA will therefore be separated by size, with the smaller pieces towards the bottom and the larger pieces towards the top.

STEP 3

The DNA fragments are separated according to their size using gel electrophoresis. For this step, you will need plenty of space to place the four "paper gel lanes" provided side by side as follows:

Gel lane 1:	Crime scene DNA
Gel lane 2:	Victim's DNA
Gel lane 3:	Confessed murderer's DNA - Suspect 1
Gel lane 4:	Suspect 2's DNA

Sort the fragments generated from each DNA sample and place them on the corresponding paper gel lane in decreasing size, according to the DNA size standard. For example, place a 7 base pair size DNA fragment at the corresponding location on the gel of a 7 base pair DNA fragment of the DNA size standard, and so on until all of the fragments have been distributed down the gel lane.

Note: *In a typical electrophoresis procedure, the gel would be stained to reveal the location of the separated fragments. However, in an actual DNA fingerprinting procedure, if the DNA were stained at this point, there would be so many fragments from each unknown DNA sample that it would create a smear consisting of undifferentiated bands spread over each gel lane.*

Denaturing DNA into single strands

The double-stranded DNA fragments that are in the gel are denatured and split into two single strands. Denaturing of the DNA can either be done by heating or chemically treating the DNA in the gel. During this process, the DNA double helix unwinds, breaking the hydrogen bonds to form single DNA strands. The gel with the denatured single-stranded DNA fragments is then applied to a sheet of nitrocellulose paper, then heated to permanently attach the DNA to the sheet.

STEP 4

Remove the clear connectors (hydrogen bonds) and pull apart the double-stranded DNA fragments sorted on the gel, into single fragments. This step exposes the nucleotide bases allowing them to bind to corresponding complementary bases that make up the radioactive probe used in Step 5. For simplicity, remove the 5' to 3' single stranded DNA fragments from each lane.

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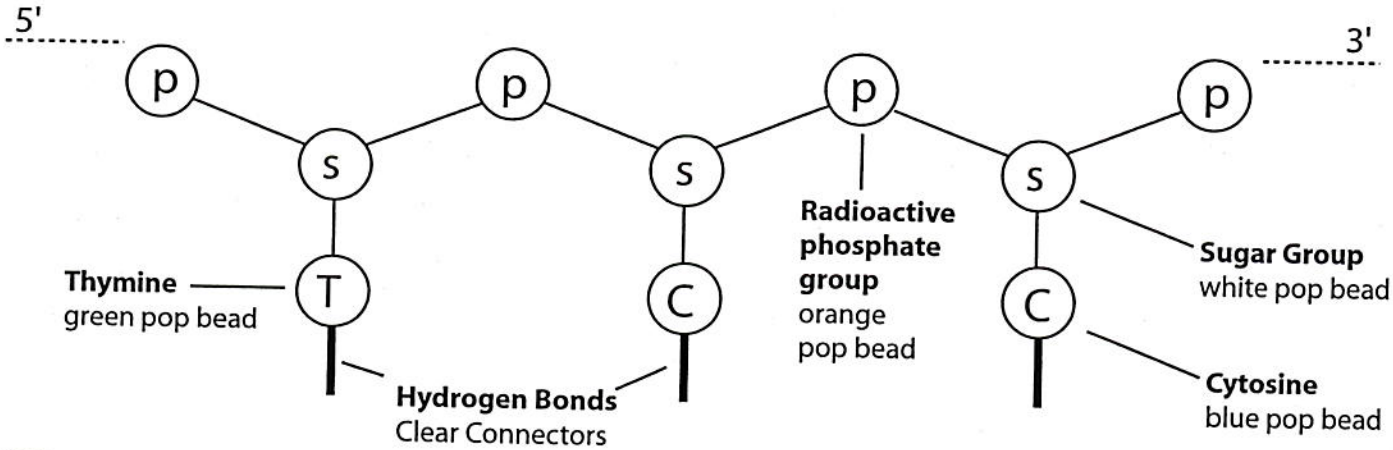
Hybridizing the DNA

In order to distinguish one suspect's DNA from another, a short DNA strand with radioactive phosphate groups, called a probe, is used to locate specific DNA sequences in each unknown DNA sample. The nitrocellulose filter paper containing the single-stranded DNA fragments is treated with a radioactive DNA probe. The probe finds and binds to a complementary sequence in one or more RFLPs bound on the nitrocellulose filter paper. To reduce the possibility of a mistaken identity, forensic scientists may use several different probes to locate various complementary DNA fragments in each unknown DNA sample.

Autoradiography

An x-ray film is then placed over the nitrocellulose filter paper, to expose the film at the position where the probe has hybridized with complementary DNA sequences in the RFLPs. The position where the probe has binded on the RFLPs will be detected as dark bands on the x-ray film. The banding pattern on the x-ray of the RFLPs that hybridize to the probe represent that individual's unique "DNA fingerprint". The RFLP pattern of two or more DNA samples is then compared for similarities.



STEP 5

Assembling the probe

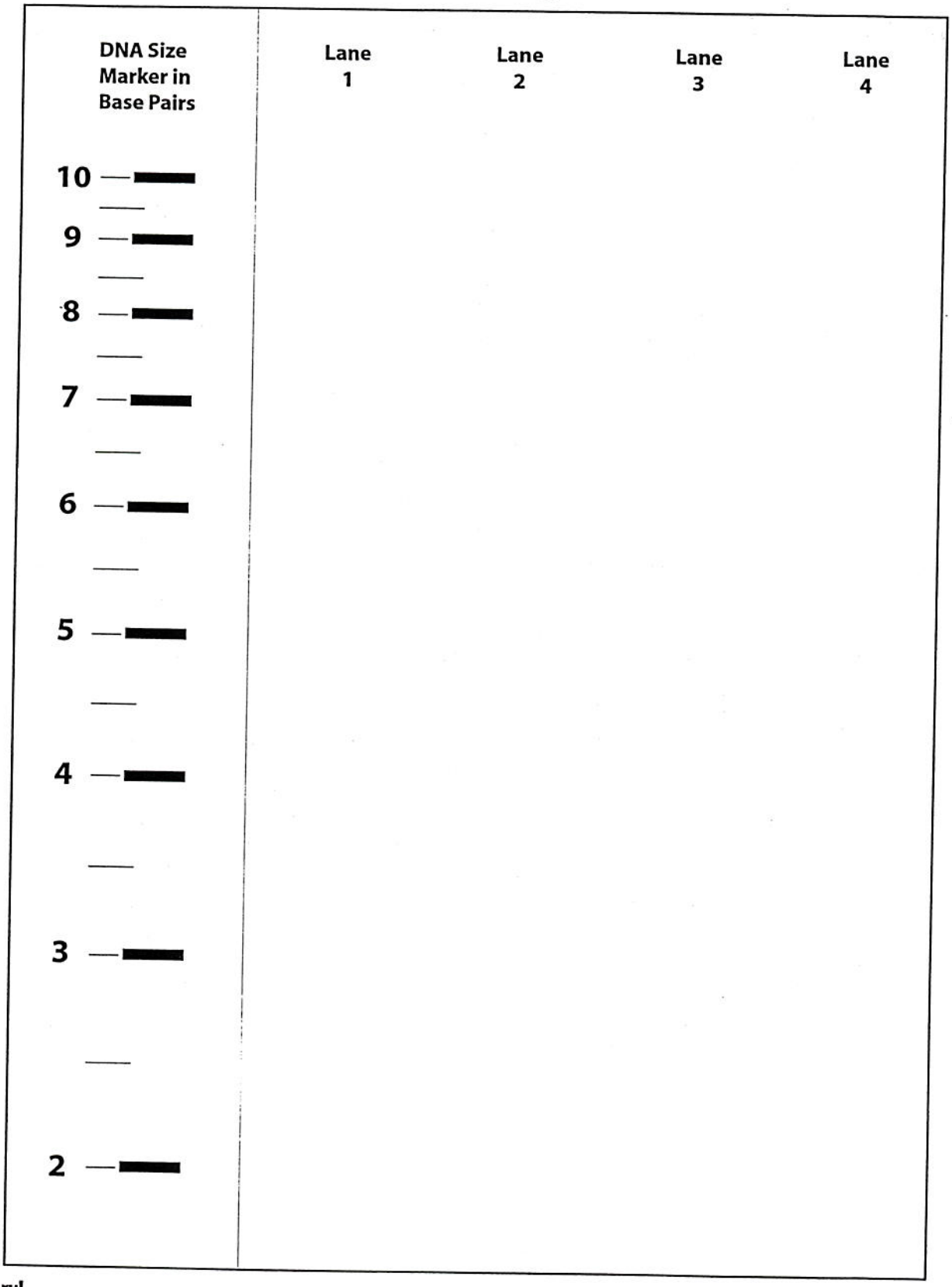
Use the components of the single-stranded DNA fragments, removed from your paper gel in the previous step, to assemble 9 individual single-stranded probes with a TCC base pair sequence. Be sure to use orange pop beads for each phosphate group to indicate that it is radioactive.

Pass the probe over each DNA fragment on the gel to locate any complementary DNA bases. When you locate complementary DNA bases, bind the probe onto the DNA fragment using the clear connectors, which represent hydrogen bonds. Be sure to leave the DNA fragments that have hybridized to the probe on their original position on the gel.

Recording Observations

The hybridized DNA fragments on your paper gel represent each individual's DNA fingerprint. Record your results in the autoradiogram diagram. Using a black marker, fill in the autoradiogram transparency indicating the exact location of each fragment and the unique DNA banding pattern of each DNA sample.

Autoradiogram



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Questions

1. Based on your autoradiography results, which suspect is the murderer? Explain your answer.

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2. What would the resulting autoradiogram look like if you performed the DNA fingerprinting procedure but skipped:

a) digesting the DNA with restriction enzymes

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b) gel electrophoresis

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c) separating the DNA into single strands

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d) transferring the single-stranded DNA to nitrocellulose paper

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e) hybridizing the DNA

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f) autoradiography

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3. Discuss with your teammates the validity and controversial aspects of DNA fingerprinting.

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4. Before DNA fingerprinting was developed, paternity testing was based on blood type. This is because an individual's blood type is based on the blood types of his or her parents. Since many people have the same blood type, this type of test can only be used to exclude possible biological parents, but not to actually prove who the parents are. DNA fingerprinting, on the other hand, can prove familial relationships with a high degree of certainty.

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DNA Size Marker in Base Pairs

Paper Gel Lane 1
Crime Scene DNA

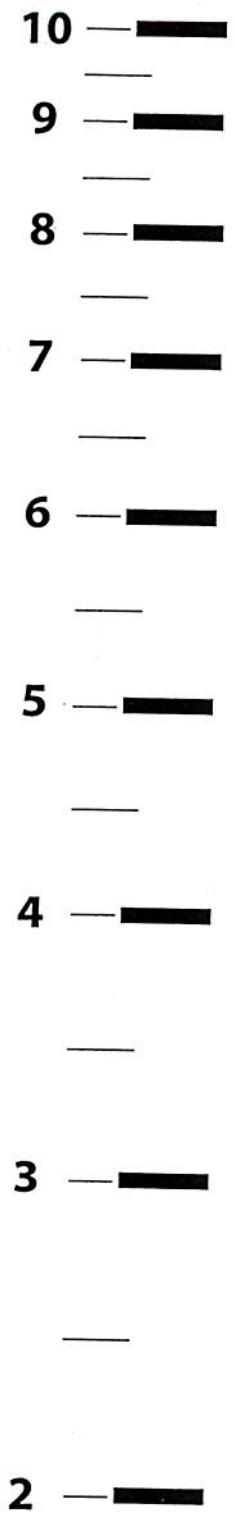
DNA Size Marker in Base Pairs

10
9
8
7
6
5
4
3
2

Paper Gel Lane 2
Victim's DNA

DNA Size Marker in Base Pairs

Paper Gel Lane 3
Confessed Murderer's DNA



DNA Size Marker in Base Pairs

Paper Gel Lane 4
Suspect 2's DNA

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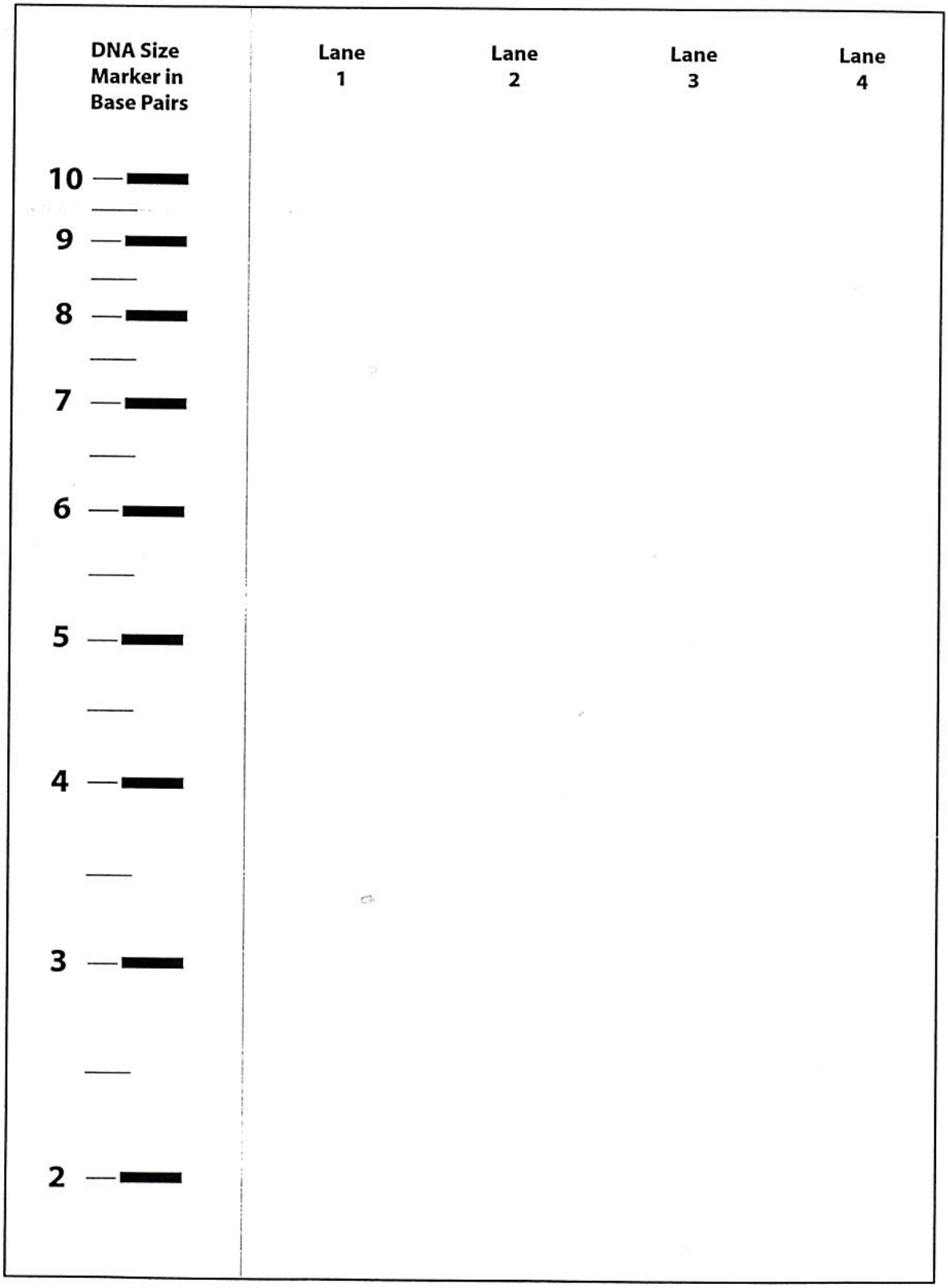
Autoradiogram



DNA Murder Mystery!