# Where's the "CAT"?
## A DNA Fingerprinting Simulation

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## INTRODUCTION

"Where's the CAT?" was developed to help students better understand the techniques used in DNA profiling. Electrophoresis, restriction endonucleases, Southern blots, variable number tandem repeats (VNTRs) and restriction fragment length polymorphisms (RFLPs) are all involved in this exercise to help determine the parentage of an unborn (or newborn) individual. Although simplified, the exercise demonstrates the use of DNA fingerprinting in modern forensic science and allows students hands-on experience in the step-by-step procedures involved. This exercise has been successfully used with science students in grades 7-12.

Pages 4-7 contain the original "CAT" text. This exercise appeared in modified form in the December 1991 issue of The Science Teacher. The editorial staff of the journal felt that the topic of rape was too emotional so it was modified to read:

> The activity simulates the following situation: A married couple (the woman is infertile) arranges with a surrogate to have a baby. The surrogate mother is artificially inseminated with the man's sperm. When the surrogate mother gives birth to the child, she decides that she wants to keep it. She claims that the child's biological father is not the sperm donor, but her own husband. The case is taken to court to decide custody. Genetic testing is done to determine the true biological father.

If you with to use the above scenario, change all references to "suspect" to read "sperm donor". (Results will be that the husband was the biological father in either scenario).

There are many other scenarios that could be developed using the same sequences. We have found that our students have responded will to the rape scenario because there is a "happy" outcome when the husband is identified as the biological father, rather than the rape suspect. In fact, there is often cheering as the last probe is put in to place and the class realizes that the husband is the father. Using the surrogate scenario, there is no "clean" resolution: two families are in conflict and someone is "hurt" no matter what the outcome. Nevertheless, this scenario offers many opportunities for excellent ethical discussions, and paternity cases are a major use of this technology. It should be noted that the forensic applications of DNA profiling provide a natural bridge to the topic of population genetics and applications of the Hardy-Weinberg law.

# Where's the CAT?

## An Out line of Essential Background



Assuming no previous background regarding DNA, the following outline is suggested for effective use of this activity. Some of the material should be presented before the activity (I-V or VI), while some can be presented after the activity (VII). The depth in which these topics should be covered will vary depending on the level of the students and the teacher's objectives in including this activity.

I. Where is DNA found?

II. What is the structure of DNA?
   - A. Components
     1. Sugar-phosphate "backbone"
     2. Bases
        - a. Purines: adenine and guanine
        - b. Pyrimidines: thymine and cytosine
   - B. "Double strandedness"
   - C. Complementary
   - D. Code: what is a gene? What is a chromosome?
   - E. Individuality: number and inheritance of genes, recombination (A simple mathematical calculation demonstrating random shuffling of the 100,000 genes of each parent and the chance of a random fertilization yielding the identical set of genetic traits puts the chance of another identical DNA fingerprint at about 1 in 10 billion, with the exception of identically twins. A discussion of linkage on chromosomes and alleles is appropriate to modify this number if the level of your students warrants this).

III. Restriction Enzymes
   - A. Origin and function
   - B. Naming
   - C. Recognition sites: specificity and palindromes
   - D. Types of cuts: blunt and sticky
   - E. Applications
     1. Mapping
     2. Cutting long sequences to shorter pieces
     3. Isolating selected genes

IV. Gel electrophoresis
- A. What it does
- B. How it works: charges and size
- C. Fine points: types of gels and other factors that affect separation
- D. Agarose separation of DNA
    1. Charge of DNA
    2. Specificity
    3. Rate of movement
        a. Loading dyes
        b. Relationship of fragment size to rate of migration
    4. Staining the DNA
    5. Use of a standard "ladder"

V. The rest of the story: Genetics
- A. What is a gene?
    1. Alleles and dominance
    2. Meiosis: segregation and independent assortment
- B. Sexual reproduction
    1. Two parents
    2. Crossing over: recombination
    3. Number of gametes made by each parent

VI. Forensic applications
- A. VNTRs and RFLPs
- B. Increasing specificity: blots, hybridization and probes, auto radiography
- C. Sample sources
- D. Hardy-Weinberg and population genetics

VII. Other applications of these procedures
- A. Genetic screening: diseases
- B. Paternity and family relationships
- C. Gene mapping
- D. Recombinant DNA

# Where's the CAT?



DNA profiling is now being used in forensic cases as a method of assessing probability of suspect involvement in crimes where DNA samples are available. DNA may be extracted from relatively small samples of cells, such as a blood stain the size of a nickel (about 2 drops) or a semen stain the size of a dime. When performed under properly controlled conditions and interpreted by an experienced forensic scientist, such profiling can link a suspect to a particular incident with compelling accuracy. This simulation activity allows students to work through the theory of DNA profiling and to grapple with some analytical and ethical questions. It can be used to teach the principles of restriction enzyme digestion, gel electrophoresis, and probe hybridization. Simpler concepts such as base pairing can also be reinforce as students work though the activity.

DNA profiling involves four basic procedures. Once the DNA has been isolated from the tissue sample (a procedure not included in this simulation), it is then cut with a restriction enzyme. Such enzymes recognize a specific sequence of bases on the DNA and act as molecular scissors to cut the DNA strand within the recognition sequence. Our enzyme is known Hae III, which recognizes the sequence GCC and cuts the DNA between the center G and C, leaving one fragment that ends with GG and another fragment that begins with CC. Within the human genome, this particular sequence will reappear at many points. Because each person inherits a unique combination of sequences, the number and size of fragments created fro each person's DNA should be as individualistic as a fingerprint. These different size fragments are the result of restriction fragment length polymorphisms (RFLPs), i.e. within a population, there will be many forms or lengths (polymorphs) in which the restriction fragments may appear as a result of individual differences in the genetic sequences.

The next problem is to separate the fragments according to size. This is accomplished by gel electrophoresis. The digested DNA sample is place at one end of an agarose gel and electric current is applied. Because DNA is a negatively charged particle, it migrates toward the positive electrode in the gel chamber. The agarose molecules in the gel act as a sieve, allowing the smaller DNA fragments to move through the spaces between agarose molecules faster than the larger, more cumbersome fragments. A standard "ladder" of fragments of known sizes is run with the unknowns for comparison and as a check that the gel is uniform and permits each unknown to separate smoothly. At this point, if the DNA were stained, there would be so many fragments from each unknown sample that the DNA would appear to be an undifferentiated smear across the

gel. In order to distinguish between the DNA of one individual and that of another, there must be a way to identify a specific pattern of inherited alleles.

In an attempt to solve this problem, the DNA smear is transferred to a nylon filter to prevent further movement and degradation of the DNA fragments. In the process of transferring the DNA from the gel to the nylon filter, the double strands are separated, and the filter contains only single stranded DNA, to which a complementary sequence of DNA could bind. (NOTE: this simulation uses only a single strand of DNA throughout the restriction analysis procedure for purposes of simplification.) Radioactively labeled sequences of single strand DNA have been developed that recognize particular sequences of DNA that are not thought to be transcribed, but which do appear in a randomly repeated fashion throughout the genome. These fragments are called variable number tandem repeats (VNTRs); These fragments have been developed to identify particular sets of alleles, and extensive work is now being done to quantify the frequency of particular VNTRs within a population. Each individual should inherit one allele of a particular size and containing a VNTR from one parent and a homologous but not necessarily identical allele with the same VNTR from the other parent. If several labeled DNA strands of particular sequences (called probes) are allowed to combine with the DNA sample of the filter, one probe at a time, the resulting combinations of labeled fragment should be able to distinguish the DNA of one individual from that of another.

This is intended as a group activity, in which the class will be split into several groups, each of which will contribute data to the final "auto radiograph". Each group will need about 4 cm of magnetic strip, except the group doing the standard, which will need about 20 cm (these are flexible strips of magnetic material that can be cut with scissors and can be bought at most craft stores); In addition, each group will need tape, scissors, a pen, copies of nucleotide sequences, a 2 cm by 20 cm strip of brightly colored construction paper, and a copy of the directions. The teacher will need a large sheet of poster board and five #6 envelopes to use in assembling the DNA fragments on the "auto radiograph".

The activity simulates the following simulation. A woman was raped. Later she is found to be pregnant, but does not know whether she is pregnant by her husband or the rapist. She turns to molecular biology for more information.

Each group of 2-4 students should be assigned to one sample. A minimum of five groups can be assigned. Students can work separately if materials and time permit. To make the simulation manageable, each simulation base represents approximately one thousand bases (one kilobase) that would be found in an actual DNA profile.

To simulate the restriction digest, each group must first cut out the strips of DNA sequences, and then tape together the strips representing one sample of DNA, being sure to match and obscure the subscripts as the sample is assembled. Next, scan the sample strip for the probe sites: CAT. Wherever the sequence CAT appears, tape a 1 cm piece of magnet to the back of the strip. Next, mark the sample strip at the recognition sites for the restriction enzyme Hae III (GGCC). Then cut the strip all the way across between the center G and C of each restriction site. (NOTE for teachers: Each group with a sample should have six smaller fragments at this point; the standard should yield eight smaller fragments. Provide each group with a small-labeled envelope. This can represent a well in the agarose gel).

To simulate gel electrophoresis, use a large (at least 62 cm x 74 cm) sheet of poster board preferably in a contrasting color, on which to assemble the fragments resulting from the restriction digest. The standard should be placed first. Exact distances from the origin in the "well: are not important, as long as all fragments of the same length are placed the same distance from the well, and the larger fragments are placed closest to the well with the smaller ones being placed farther away in descending order beneath the well. Use the envelope that contained the standard fragments to represent the well. Place the 20 base fragment at least 5 cm below the well, the 18 base fragment at least 5 cm from the first fragment, and so on until all eight fragments have been distributed down the column. Tape the fragments in place. Place the envelope for the mother's samples to the right of the standard sample envelope, and then repeat the placement of fragments in another lane parallel to the standard lane. Note that the mother's 20 base fragment should be the same distance from its well as the standard 20 base fragment is from its well. The mother's DNA yields a 9 base fragment; this should be placed at a distance intermediate to the 10 and 8 base fragments of the standard. Continue placement of samples in the same manner, moving to the right across the poster board in the following order: husband, suspect, and child. When complete, each sample contains six different fragments. The fragments of one sample are the same size as the fragments of the other samples, but they differ from those of the other samples in their specific base sequences.

These fragments must next be differentiated from one another; the sequences in a real gel would not be known. Construct DNA probes by cutting 1 cm by 2 cm rectangles from the brightly colored construction paper. The standard group will need eight rectangles and the sample groups will need two each, for a total of sixteen rectangles needed fro the class. The sequence "GTA" should be written on each rectangle (a template for photocopying is included) and a 1 cm magnet should be taped to the back of each. These DNA probes will be used to identity specific alleles. The probe sequence (GTA) is complementary to the VNTR sequence (CAT).

With a probe in hand, scan the standard and position a GTA probe on each VNTR site. Notice that each fragment in the standard has a VNTR site and can be labeled with the probe. (This procedure of combining DNA from two sources is call hybridization). Repeat this procedure for each sample. Notice that only tow fragments from each sample will bind with the probe. Each labeled fragment represents a part on one chromosome of a homologous pair. To simplify visually, remove all unbound sequences form the "gel". Analyze the banding patterns according to Mendelian principles: the child inherited one allele from each parent. In our example, the mother could only have donated one of the child's labeled alleles. Which man is more likely to have donated the other allele?

Further discussion could deal with various situations. What if the biological father were the rapist? Would abortion be justified here? What if the samples represented two suspects in the rape instead of the husband and suspect, and a stain from the woman's clothing instead of a fetus? Would this be sufficient evidence to convict a suspect of the crime? What limitations can be seen in these procedures?

This activity can be completed in one to three fifty-minute periods, depending on preparation and discussion time. It can be done with any life science class from middle school to advanced placement biology. Other concepts that can be added include the reading of DNA sequences from 5' to 3' ends, the use of multi-locus probes, nucleotide structure (as the production of labeled probes is discussed), and the use of other restriction endonucleases. Other situations could be developed if a rape is inappropriate for discussion. For example, there is a British immigration case where a child was temporarily denied admission to Great Britain from Ghana until it was shown by DNA technology that he was, indeed, the child of his mother rather than of his aunt as was alleged.

# Where's the CAT? STUDENT DIRECTIONS



Each group needs:
4 cm magnetic strip (standard group needs 20 cm)
1 paper base sequence
1 strip of fluorescent paper (2 cm x 20 cm)
1 envelope (letter size)

Before coming to class each group should:

1. Label the envelope with the name of your group
2. Cut our your sample DNA strips, as assigned to your group. Tape together the strips for your sample, obscuring the subscripts, so that you have one long paper strip of bases.

3. Cut your strip of magnetic material into 1 cm chunks.

4. Each sample group should prepare 2 "probes" by cutting 2 rectangles of fluorescent paper, each 1 cm x 2 cm, and writing "GTA" on each rectangle in large black letters. The standard group should prepare 3 probes, using these directions. (Note: There is a "GTA probe template" included to be xeroxed as an alternative to this step).

In class:

1. Scan your DNA strip until you find "'CAT". Tape one 1 cm magnet to the back, with the magnetic side toward he paper, at each place where you find "CAT".

2. Tape a magneto the back of each GTA probe with the magnetic side toward the paper.

3. Scan your DNA strip until you find "GGCC". Cut across the strip between the center G and C; you will be forming a fragment that ends with GG and another that begins with CC. (HINT: each sample should have 5 of these sites, giving 6 fragments. The standard contains 7 of these sites, giving 8 fragments). Count the number of bases in each fragment and write that number on the fragment.

4. Place all fragments in your labeled envelope and wait for your teacher to give further directions.

Standard

$_{1}$CCAAGACATTATGCAGATGGCCAATAGACATTACGG$_{2}$

$_{2}$CCATACCAGAGGCCCAACATGGCCAAACACACCC$_{3}$

$_{3}$ATCAGGCCATGGCAGACGGGCCATACGGCCATGG

Mother

$_{1}$CCTAGACGGCCAGGCACAAGCCAGGCCATGGCCAC$_{2}$

$_{2}$ATCAGTTAGACCGAGGCCGAATCAGGCCTTATTGCAGG

Husband

1 CCGAGGCCAGGGTATACCGGTATAGGCCAATTTGGCC

2 GGCATGGGCCGATACAGCCGATGGCCATATAGGGGG

Suspect

1 CCGGTACATTACCAGGCCAAGGATACGGCAAGCAGG 2

2 CCTTCATGGCCAAGGCCTTAGCACGGGCCAATGACGG

Child

1 CCACATCAGTTAGACCGAGGCCAAGGCCAACCGACG 2

2 GCAAGGCCCGACAGGCCAAAGACGGCCATATAGGGGG

# Template for DNA Probes

(SHOULD BE COPIED ON BRIGHTLY COLORED PAPER, OR COLOR MAY BE ADDED AS STUDENTS MAKE PROBES)

| | | | | | |
|---|---|---|---|---|---|
| GTA | GTA | GTA | GTA | GTA | GTA |
| GTA | GTA | GTA | GTA | GTA | GTA |
| GTA | GTA | GTA | GTA | GTA | GTA |
| GTA | GTA | GTA | GTA | GTA | GTA |

# Some Qualifying Comments



There are some limitations to this activity and some details that warrant comment.

This simulation does not include a step in which the DNA is denatured, or made single-stranded. This is accomplished in the laboratory by treatment with an alkali solution before a blot is made of the gel.

We have only generated a few fragments for each sample. In reality, there would be many thousands of fragments of varied sizes generated by a restriction enzyme digest because of the size of the human genome. These would vary almost continuously so that, if a gel were simply stained for the presence of DNA, a smear would appear from the well to the opposite end of the gel for each sample and on one's sample would show any difference from anyone else's. This is why probes must be used.

The DNA must be denatured before preparation of the blot because the single-stranded probes must hybridize with the sample fragments on the blot. Single-stranded DNA can only bind to fragments of different sized in different individuals because of VNTRs (see additional background). What we have done is generate fragments in our simulation that are the same size for each individual, and some fragments even show similarity in sequences. The point should be made that, just as our "simulated gel" shows little or no difference in the DNA of different individuals before application of the GTA probes, a real gel will not be able to differentiate individuals without application of specific probes, because there is no other convenient way to tell the sequence of each fragment. The fragment sizes can be calculated by comparison with a standard, but there are even limitations to this calculation. Fragments of similar but not identical size may separate together, or so close together that they cannot be distinguished as being separate. In fact, some VNTR alleles are so similar in size that it is difficult if not impossible to differentiate them. This must be considered in choosing appropriate probes to be used.

# Additional Background



The principle behind DNA profiling (the name patented by Dr. Alec Jeffreys, who first developed and used this procedure), is that each person will have an unique combination of sequences throughout his or her genome, having inherited half of his genome from one parent and half of his genome from the other parent. Only identical twins would reasonably be expected to have perfect matches in their DNA sequences, and even they might show evidence of spontaneous mutations. The human genome is estimated to include 50.000 – 100,000 genes in the $6 \times 10^9$ base pairs located in the 22 pairs of autosomes, 2 sex chromosomes, and mitochondrial chromosome. When a typical human genomic DNA sample is cut with restriction endonucleases and the resulting fragments are separated by electrophoresis on an agarose gel, a smear of SNA fragments will result. There is so much DNA yielding so many fragments that differ so slightly in number (which is the factor that will affect separation on the gel) that they cannot be separated into discrete bands containing similar DNA fragments. Rather, they form a continuum of pieces smeared across the gel, and everyone's smear will look the same if simply stained for DNA. An additional step is necessary to identify particular allele combinations in an individual's DNA within this smear.

It is estimated that 98 – 99% of our DNA is not translated into protein products. These sequences "in between" the expressed genes are known as **introns** and have been shown to contain many repeating short sequences that vary in number from person to person but have no phenotypic effect. These are called **variable number tandem repeats or VNTR's** for short. If a VNTR repeats 20 times in one homozygous person's allele but only 5 times in the allele carried by another homozygote, then these two alleles should be found in different locations on a gel. Using a radioactive probe designed to find this particular VNTR, the samples of these two individuals should be easily identified at different locations on a **Southern blot** made from this gel. The offspring from these two individuals should have one allele of each type, since VNTRs are inherited according to Mendelian principles. Obviously, all offspring of these two individuals will have the same "DNA fingerprint" using only this single VNTR. Forensic applications will involve the use of several VNTR probes, and there are usually many alleles in the general population for any single probe. The different sized fragments that will result from a restriction digest of a DNA sample due to the presence of VNTRs are called **restriction fragment length polymorphisms, or RFLPs**. The chance of a particular combination of RFLPs occurring by random chance in one individual is calculated by multiplying the chances of occurrence of the individual RFLP alleles found in the sample. For example, if our RFLP with 20 repeats has an allele frequency of 1/10 and the RFLP with 5 repeats has an allele frequency of ½, then the heterozygote combination of the two alleles by random chance will occur in 1/20 of the population. Other VNTR probes would be multiplied into this equation, thus decreasing the chance that a particular combination would occur by random chance in any particular individual.

# Controversy, Applications, and Ethics



Recent controversy has centered around Hardy-Weinberg-related arguments regarding the randomness of mating in human populations. It is argued that members of many ethnic groups intermarry, and that certain RFLP alleles occur more or less frequently within various ethnic populations as a result. Further work is being done to establish reliable databases for reference within ethnic populations as well as within the general population of humans. Some mathematical calculations have indicated that this problem may be overstated by its proponents. Nevertheless, when lives are at stake, there are some who argue that this technology is not yet ready to be used for inclusion of a suspect but only for exclusion. Thus far, clear-cut conclusions (either inclusive or exclusive) from DNA evidence have been reached in 60-65% of cases in which it has been used. Approximately one third of suspects have been excluded to date, when DNA evidence has been used. In other cases, the defendant entered a guilty plea once it was known the DNA evidence was being used. In a significant number of remaining cases, however, the results of DNA testing were arguably inconclusive.

There are several technical considerations that may cause concern. The amount of DNA recovered from a crime scene may be only enough to run one profile. Good science requires reproducibility. There may not be enough sample for a defendant to run an independent verification. In addition, this testing costs money; there is an ethical concern when an indigent defendant could use this technology to support his innocence but cannot afford it. The quality of the DNA is also of concern. Heat, moisture, and microbial contamination are but a few of the enemies of DNA. The **polymerase chain reaction, or PCR** can be used to amplify small amounts of DNA found at a crime scene (so that the DNA from a single hair root can be sufficient for testing), but any contamination would also be amplified, possible obscuring the target DNA from the perpetrator. Crime scene investigators must be trained in proper recovery of evidence which could be used for DNA profiling. In addition, the alleles for some RFLPs may fall close together on a gel. Even with a standard or comparison sample in an adjacent lane, it may be difficult to discern one allele from another with accuracy.

The standard used to determine the size of various RFLPs is an aliquot of DNA of known composition, and it is treated with the same restriction enzymes and probes as the unknowns. Each gel runs a little differently due to minute variations in agarose concentration, voltage, buffer, temperature, etc. In addition, there might be variations within a gel due to unseen “lumps” of agarose, uneven cooling of the gel, etc., so standards are run in every third or fourth lane on a gel. These standards are commercially prepared and are carefully controlled the manufacturer. They are treated in the same way as the unknowns throughout the procedures of DNA profiling and can be used as controls since their makeup is known and the investigator knows what pattern to expect from them with the various enzymes and probes being used. The use of computers to scan the autoradiograph and measure migration distances with precision has reduced the amount of human judgment that must be exercised in interpreting the results from SNA profiling. Nevertheless there is still a good deal of judgment and experience required in assessing the quality of a give DNA sample; the quality of the gel, blot or auto radiograph; or the precise interpretation of results.

There are many other uses for this type of technology. Paternity cases can be resolved conclusively, given samples from both putative parents and proper controls. Immigration cases, including Dr. Alec Jeffreys' first publicized use of DNA profiling in England are also candidates for application of this technology. The agricultural world has used these principles in the establishment of pedigrees of livestock with certainty. Argentina has used DNA profiling in trying to reunite children of the "lost generation" with their grandparents after the terrorism of the 1950's and 1960's. This sort of technology is being used in identifying carriers of genetic disorders that may not be phenotypically obvious until after the childbearing years, particularly Huntington's disease. There is concern, however, about the use of such information. How will knowledge that one will develop a devastating disease late in life be used? Certainly individuals might decide not to reproduce and pass these genes on to another generation, but what effect might this knowledge have on the quality of life left for "the doomed"?

Of ethical concern is the potential use of samples gathered for use in the database used to determine frequency of VNTR alleles. As the human genome is characterized in progressively greater detail, might insurance companies or potential employers have access to one's DNA to check for possible susceptibility to heart disease or cancer before granting insurance or employment? Can inmates be *required* to give samples of their blood for DNA testing? (Virginia is currently embroiled in argument about whether parole can be denied unless an inmate agrees to the blood sample. The most recent court ruling is that the inmate can, indeed, be required to submit to blood sampling but that parole cannot be affected by one's refusal to provide such a sample.) Could these samples be used to develop a national or international database that might be used in assessing guilt or innocence in future crimes without other substantiating evidence? What about the problems of simple human error in labeling or processing a sample? What controls should be established to minimize or eliminate such problems? Who will oversee the training and licensing of laboratories to perform this technology? Alec Jeffreys' original procedures were developed in the United Stated by Cellmark, followed by development of similar procedures by Lifecodes. The F.B.I. laboratories have the most experience with forensic use of DNA profiling, but more and more state forensic departments are including this technology in their laboratories. Who or what agency will oversee the proper training and standard in the blossoming field? How will the general public be educated as to the pros and cons of this type of evidence? That, or course, is where the expertise of secondary science teachers will be required.

In the past year, great scientific debate has raged regarding these questions, as science comes into conflict with the legal system. The National Academy of Science established a committee, chaired by Victor McKusick of Johns-Hopkins, which has recently published a list of recommendations regarding this technology. Those recommendations include establishment of a conservative statistical ceiling for each allele at each locus in 15 – 20 homogeneous populations; mandatory accreditation and proficiency testing (under the auspices of the Dept. of Health and Human Services, with the F.B.I. and the Dept. of Justice serving as consultants, thus including the F.B.I. in the same objective standards as commercial labs); and establishment of an ad hoc expert group, called the National Committee on Forensic DNA Typing to evaluate new approaches, collect samples for population studies and to advise the course with respect

to statistical probabilities. This Committee would be affiliated with the National Institutes of Health or the National Institute of Standards and Technology with support from the National Institute of Justice and the National Science Foundation.

As debate has continued among scientists, some criticism has been aimed at our legal system. Never before has evidence been so rigorously debated. As pointed out in *Science* in the letters to the Editor (28 Feb '92), polygraph information is sometimes allowed, despite its 20-30% error rate. Ballistics and handwriting evidence have been allowed for many years, although they were validated in a time when the validation of data was not very rigorous. "Nonscience" evidence (that which is understood by the lay public!) has been accepted without any reliable criteria; for example, dog trainers can present olfactory identification by their dogs without rigorous validation. It is important that our students be given the process skills necessary to examine all types of evidence and to separate fact from opinion, which is one of the most exciting spin-offs of this simulation activity. Students need to examine these questions critically to become responsible, thoughtful, and productive adults in tomorrow's world.

# Glossary

Agarose — carbohydrate used in varying concentrations for form a matrix for gel electrophoresis for separation of DNA fragments by size; all DNA fragments are negatively charged in solution, so fragments move toward the positive electrode during electrophoresis.

Allele — alternate forms of a particular gene or DNA sequence; e.g., a gene for hair color may have either red or blond alleles, and expression will depend on the particular combination of alleles present in an individual; with respect to DNA profiling, alleles may refer to VNTRs of different numbers, i.e., one allele may contain two repeats of a short sequence while another may contain twenty repeats of the same sequence.

Autoradiograph — X-ray film that has been exposed to radioactivity from the hybridization of a blot with radioactive probe(s) to show the location of particular alleles; the radioactive probes have been allowed to "take a picture of themselves"; final product in DNA profiling; shows which alleles are present in sample(s).

DNA — deoxyribonucleic acid: genetic material in nuclei of cells; molecule from which genes are made; consists of two sugar-phosphate backbones with complementary nitrogenous bases holding the two strands together with hydrogen bonds to form a double helix.

Gel electrophoresis — process of using electrical charge to move molecules, fragments, or other materials through a matrix composes of molecules of agarose, polyacrilamide or other substances; materials will separate by size and/or charge, depending on particular substances involved.

Genome — All of the chromosomes (DNA) in an organisms; total amount of genetic information in an organisms (haploid number of chromosomes).

Hybridization — binding of two DNA strands or segments from two different sources; e.g., the denatured DNA on a Southern blot may be hybridized with manufactured probe DNA of complementary sequence.

Probe — single strand nucleic acid sequence, often labeled with radioactivity or some other distinguishing marker, used to identify particular loci on a blot.

Restriction enzymes — enzymes made in bacteria to destroy foreign DNA; used as "molecular scissors" to cut DNA at a particular site to generate small fragments of DNA for examination or recombination.

RFLP — restriction fragment length polymorphism; different size allelic fragments of DNA resulting form VNTRs or from fount mutations that affect a recognition/restriction site for a restriction enzyme.

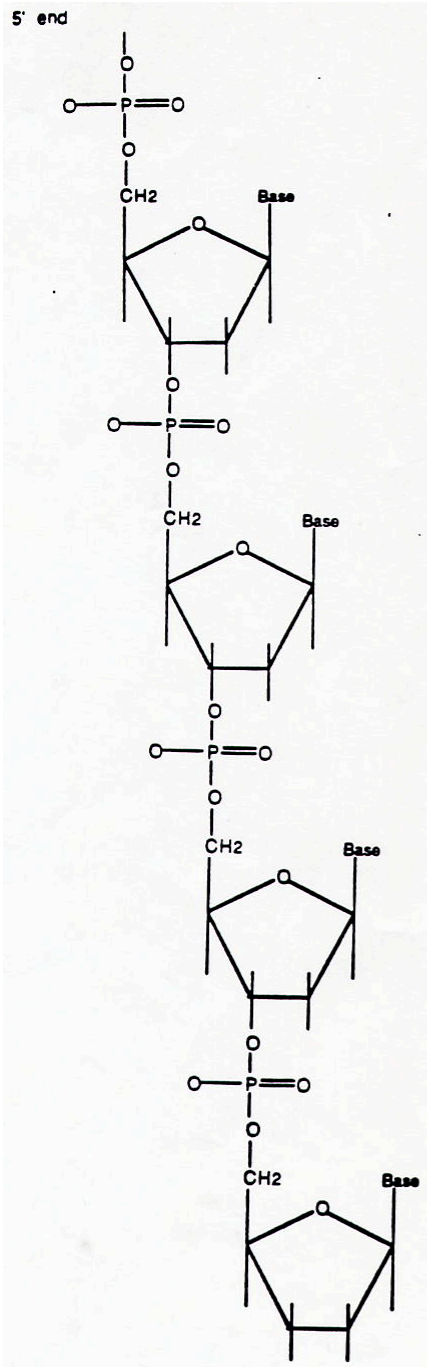
Southern Blot — transfer of DNA fragments from a gel onto a filter; stronger than a gel and can be probed more than once.

VNTR — variable number tandem repeats; short sequences of DNA which repeat in varying numbers in intron regions of genome; different alleles will be characterized by different numbers of repetitions and are inherited in Mendelian fashion; many VNTR sequences are known and can be used as probes in DNA profiling, as long as there is reasonable data available regarding allele frequency within the population.

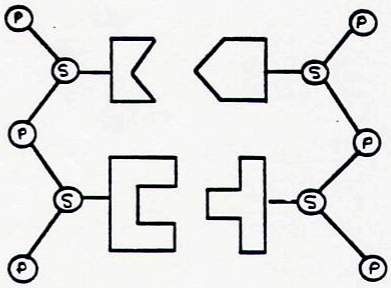
# RESTRICTION ENZYMES

## Recognition Sequences on dsDNA

| | |
|---|---|
| BamHI | 5’ N – N - G – G – A – T – C – C – N – N 3’ |
| | 3’ N – N – C – C – T – A – G – G – N – N 5’ |
| EcoRI | 5’ N – N – G – A – A - T – T – C – N – N 3’ |
| | 3’ N – N – C – T – T – A – A – G – N – N 5’ |
| HindIII | 5’ N – N – A – A – G – C – T – T – N – N 3’ |
| | 3’ N – N – T – T – C – G – A – A – N – N 5’ |
| HaeIII | 5’ N – N – G – G – C – C – N – N 3’ |
| | 3’ N – N – C – C – G – G – N – N 5’ |




THE SUGAR-PHOSPHATE BACKBONE




HOW NUCLEOTIDES FIT TOGETHER

STIONNAIRE



Complete the following, using your notes and the information that you have learned in our studies of DBA fingerprinting.

1. DNA is made of four bases in various sequences. Those bases are:
    a.
    b.
    c.
    d.

2. DNA is double stranded. Draw the sequence of bases that would be complementary to the sequence shown below.

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

3. Knowing that Hae III cuts only at its recognition site, draw arrows to show where this restriction enzyme would cut the strand shown below.

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

4. How many fragments would be created form this strand, and how many bases would be in each fragment? List the sequences of the fragments that would be created IN ORDER FROM NEAREST TO SMALLEST, as they would move from the well in a gel.

5. Once the DNA sample has been cut with the restriction enzyme, how is it separated by gel electrophoresis? (Explain why electricity is used, as well as which fragments will move the most rapidly and why).

6. After the gel has completed its separation, there is so much DNA from a particular sample that it would appear as a smear of material, rather than as separate, discrete fragments. How can the alleles of one person be distinguished from the alleles of another person?

7. What does DNA do?

8. Where is DNA found in the cell? What organisms have DNA?

9. How many genes are there in a human cell (in the human genome)?

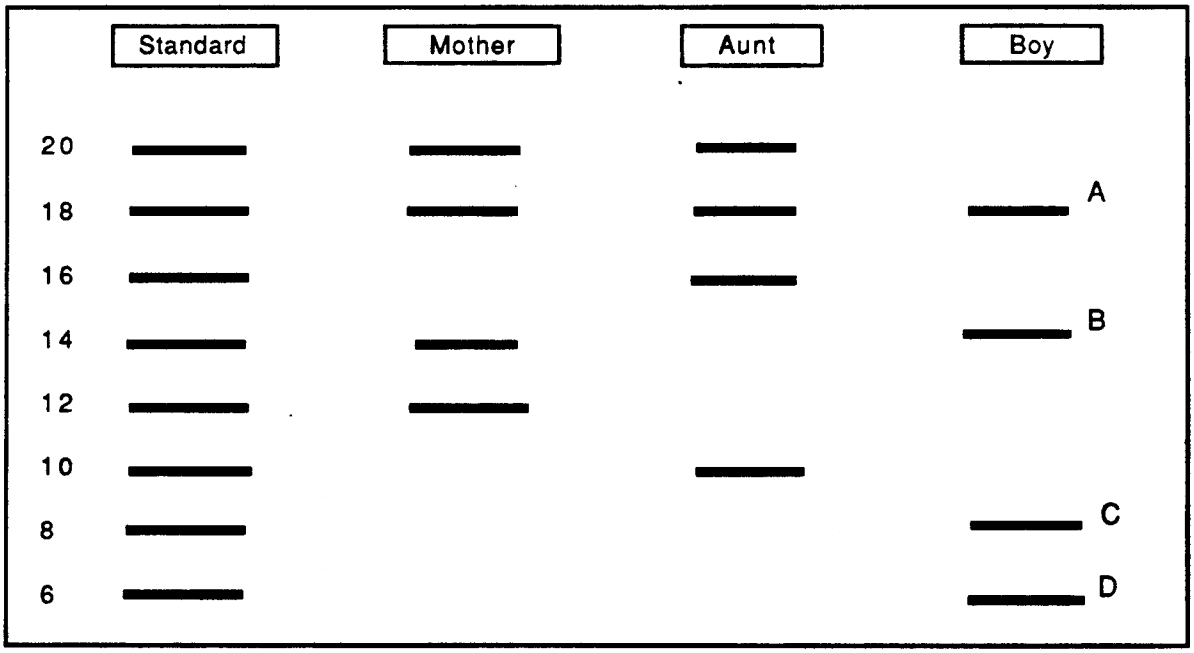
10. Why is your DNA different from that of anyone else (except if you are an identical twin)? HINT: Explain the source of your DNA and how that might be different from the source of someone else's DNA.

11. What is a DNA probe? How must it be made? What characteristics must it have?

12. Why should more than one probe be used in order to have reliable information in trying to identify an individual from a DNA profile?

13. Try the problem shown below, based on what you now know about DNA profiling.

    A boy is trying to immigrate into Great Britain from Ghana. There has been a mix-up with his records, and officials think that he is the child of an American citizen whom he claims to be his aunt. If this woman is his mother, he would not be allowed to immigrate since she is not a British citizen. The woman whom he claims as mother is a British subject, and would, therefore, give him claim to immigration rights. Molecular biology techniques are used to provide a DNA profile of each of these individuals in an attempt to straighten out the mess. The autoradiograph is shows below.




1. From whom could the boy have gotten each of the labeled fragments?
   A
   B
   C
   D

2. Can you tell with certainty which woman was his mother? How?

3. What other information could be useful to you?

4. What conclusion(s) do you draw if you know that the father's DNA provides the following labeled fragments: 16 bases long, 14 bases long, 8 bases long, and 6 bases long?

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