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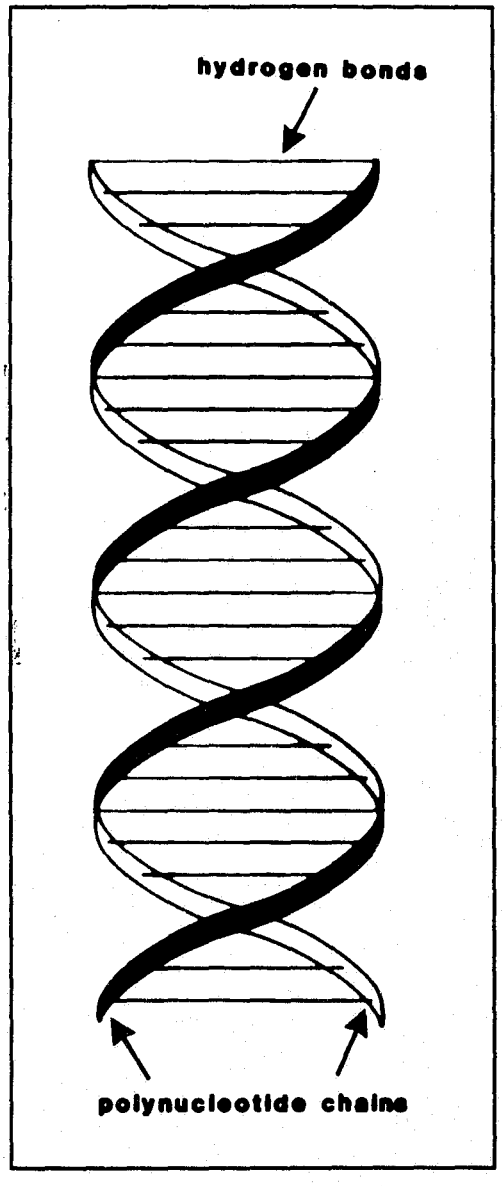
# Crime Laboratory Digest

*In This Issue*

## DNA Implementation



# CRIME LABORATORY DIGEST

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Inclusion of an article in the Crime Laboratory Digest in no way represents an endorsement or recommendation of any part of that article by the Federal Government, the Department of Justice or the FBI. Contributing authors assume total responsibility for the contents and accuracy of their submission. Questions or requests concerning an article should be directed to the contributing agency.

All submissions are subject to editorial review in accordance with the editorial policy established by the FBI Laboratory and ASCLD. The editorial staff of the Crime Laboratory Digest reserves the right to edit all articles for style, grammer and punctuation. Comments and letters to the editor are encouraged and will be published when appropriate and as space permits. These should be forwarded to:

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CRIME LABORATORY DIGEST Vol. 15, Supplement No. 1, 1988
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# Message from the Assistant Director in Charge of the FBI Laboratory

This Supplemental Issue of the Crime Laboratory Digest is provided as a means to transmit specific information to you which may be useful in your planning cycle for the implementation of DNA testing methods. A limited printing of this Supplemental Issue was mailed to approximately 300 crime laboratories in the United States in early April.

The FBI is making steady progress in its DNA research program and in its effort to transfer this important technology to the crime laboratory community. In early April, invitations were extended to directors of the laboratory systems throughout the United States to nominate individuals to participate in the Visiting Scientist Program at the Forensic Science Research and Training Center (FSRTC). This 4-month program is designed to provide the technical resources to address the validity and reliability issues associated with DNA testing as quickly as possible. Upon completion of the collaborative research project, participants will have developed a proficiency in the test methods and gained valuable experience to apply to DNA implementation efforts in their individual state and local laboratories.

To assure maximum benefits from the Visiting Scientist Program, we requested that sponsoring laboratories be fully committed to DNA implementation as indicated by appropriate equipment inventories and appropriate authority to use the radioactive isotopes employed in the DNA probes. The information in this issue is intended to help further clarify these requirements. An attempt was made to express these requirements in "generic" terms, that is, in terms that can be readily modified and tailored to an individual laboratory's needs, based upon existing facilities, examination workload and anticipated personnel to be committed to DNA testing. They should be considered as a guide for planning purposes and not necessarily a "shopping list." You are encouraged to consult with individuals experienced in DNA test procedures and discuss your specific requirements.

For your additional information, a curriculum is being developed for a DNA training course for state and local laboratory personnel which we expect to begin offering in the late Fall of this year. You will be advised as soon as the program is finalized and student applications can be submitted. The program will recommend academic background criteria for student candidates, including recent participation in a college level course on molecular biology as well as a bibliography of recommended readings to prepare for the course. A brief description of the recommended course content and an abbreviated bibliography is in this issue.

From 5/31-6/2/88, a seminar on DNA technology is being sponsored at the FSRTC. Attendance is limited at this conference due to housing constraints at the FBI Academy facility. Participants will include key forensic and medical researchers from academia, the private sector and the international crime laboratory community. Two important topics to be addressed at this meeting are the establishment of DNA standards within the forensic science community and automated data files containing DNA identification profile information. Some states have already passed or proposed legislation which provides for the collection of blood standards from convicted sex offenders. A data system containing DNA profile information from semen or other biological evidence recovered from the victim or scene of a sexual assault would permit cross comparisons to link similar crimes within the same jurisdiction or among several jurisdictions. Clearly, it would be advantageous to law enforcement on a national scale to coordinate the development of such systems with the establishment of appropriate controls and standards to permit the effective exchange of DNA identification profiles. To accomplish this, the community must agree upon standards which provide a common language and thereby facilitate the exchange of critical investigative information and, at the same time, build flexibility into the system to accommodate changes as this technology continues to evolve. The success of this effort will depend heavily on a

strong professional commitment to the needs of law enforcement along with a spirit of cooperation and mutual support within the forensic community. A report will be prepared on the events of the May-June seminar for presentation at the Las Vegas annual meeting of the American Academy of Forensic Sciences. I hope this information is useful to you, and I encourage any comments or questions you may have. It is our goal to identify the means for the full realization of DNA technology in the forensic community and to facilitate its implementation in any way possible.

**ROGER T. CASTONGUAY**

# A Primer on the Methods Used in the Typing of DNA

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## STRUCTURE OF DNA

Deoxyribonucleic acid (DNA) is an organic substance found primarily in the chromosomes that are structures within the nuclei of cells. The DNA within any chromosome is composed of two strands, wound about each other in a helical fashion. Each DNA strand is a polymer, composed of molecules, called nucleotides. Four different nucleotides are found in DNA, deoxyadenosine monophosphate (A), thymidine monophosphate (T), deoxycytidine monophosphate(C), and deoxyguanosine monophosphate (G). It is more convenient for the following discussions to refer to the nucleotides solely by their letters, A, C, G, and T.

A single strand of chromosomal DNA is a chain composed of hundreds of thousands of these letters. Because the order of the four letters can vary from one position to the next in the chain, an almost infinite variety of letter sequences can be developed. Chromosomal DNA, as was stated, is composed of two strands, and these two strands associate with each other in a certain way that is governed by the chemical properties of the letters in each strand. The letter A in one strand will associate only with the letter T in the other strand. Likewise, the letter C in one strand will only associate with G in the other strand. A short sequence of double-stranded chromosomal DNA might look like this:

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

If the sequence of letters in one strand is known, the sequence in the other strand can be deduced. The association of one DNA strand with another is not strong. If the DNA is heated to near the boiling point of water, the strands will separate. When the DNA cools, the two strands will line up in the proper way and reestablish their association. This property of DNA is important in understanding how certain sequences are detected in the DNA.

## CHROMOSOMES AND HEREDITY

The nucleus of every cell in the human body contains 23 pairs of chromosomes for a total of 46. The exceptions are the sperm cell and the female ovum (egg cell), which contain only 23, unpaired, chromosomes each. When a sperm cell fertilizes an ovum, the cell that results (zygote) will possess 46 chromosomes, 23 from the father and 23 from the mother. Since the chromosomes are the cellular repositories of DNA, one-half of the DNA in a person is of paternal origin and one-half is of maternal origin.

## REPETITIVE SEQUENCES IN DNA

Many of the letter sequences in chromosomal DNA code for the production of proteins that are required for the development and maintenance of life. These functional sequences are called genes, and always are inherited in pairs. One member of a gene pair is received from each parent.

All the letter sequences in DNA do not code for the production of proteins. Within the letter sequences that intervene between the protein coding regions can be found short sequences of letters that are repeated multiple times. Figure 1 illustrates an imaginary repeat sequence in DNA. The two double-stranded sections of DNA shown represent the multiple repeat regions from each member of a chromosomal pair. The DNA from one chromosome contains six repeats of the sequence -C-A-T-; and the DNA from its partner chromosome contains three repeats of the same sequence. The six repeat sequence was inherited from one parent and the three repeat sequence from the individual's other parent.

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

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

*Figure 1. Two double-stranded DNA sections.*

## RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

The presence in DNA of multiple repeats of letter sequences is the foundation on which rests one of the methods by which body fluid and tissue specimens can be genetically characterized. The characterization is based on a determination of the lengths of the repeated sequences harbored in specific areas of an individual's DNA. The length of a section of DNA that contains the repeated sequences is a direct function of the number of times the sequence is repeated. To determine the length of the repeat sequence present in each of the chromosomes, the repeating units must be cut out of the DNA strands and separated on the basis of their length. The repeat sequences are removed from the DNA strands through the use of special enzymes called **restriction endonucleases**. These enzymes, which are obtained from bacteria, will cut through both strands of DNA only when a certain recognition sequence of DNA letters is present. For the strands of DNA shown above, assume that the endonuclease will cut DNA wherever the sequence -G-A-T- occurs. Because the -G-A-T- sequence appears on both sides of the repeated sequence, these sequences will be freed in their entirety from the long strands of DNA upon enzymatic digestion. The repeated sequences that have been released by restriction endonuclease digestion (Figure 2) are termed restriction fragments. Length differences in the DNA that can be detected following restriction digestion are termed restriction fragment length polymorphisms (RFLP).

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

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

*Figure 2. Fragments of DNA released by enzyme digestion.*

Before the fragment lengths can be measured, the fragments must be separated from one another on the basis of their size. The separation of DNA fragments by size is accomplished by a technique known as electrophoresis. The restriction-digested DNA is placed into a gel that acts like a molecular sieve when the DNA fragments are pulled through it by an electrical field. During electrophoresis, the smaller fragments will move farther through the gel than can the larger fragments.

The behavior of the restriction fragments during electrophoresis is shown in Figure 3. The greater length of the larger fragment causes it to pass more slowly through the sieving gel than the smaller fragment. After about 16-20 hours of electrophoresis, the shorter fragment will have moved farther down the gel than the longer fragment.

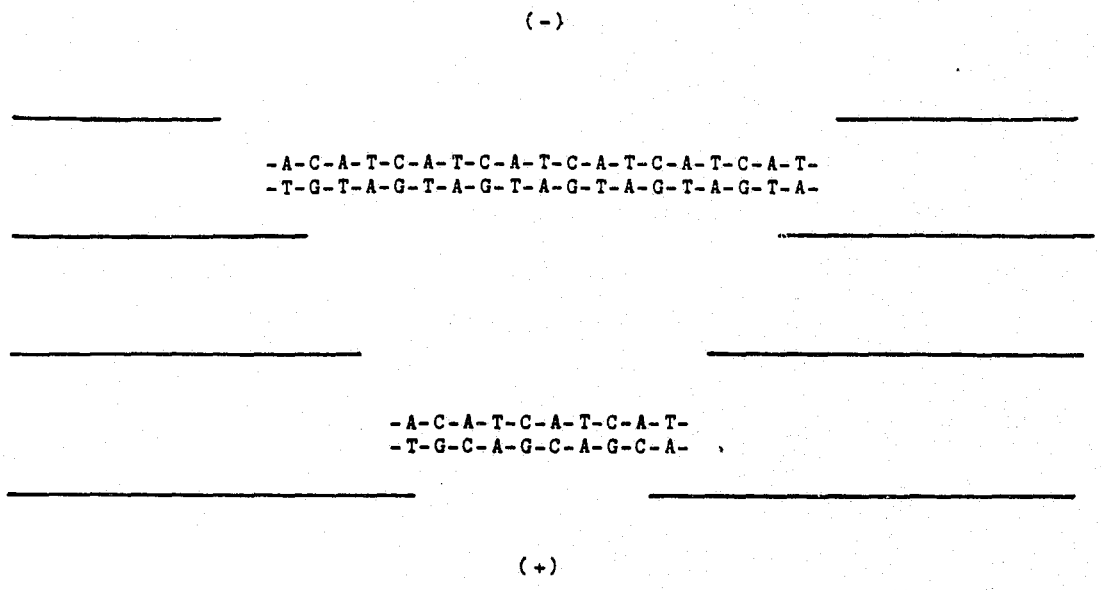


*Figure 3. Illustration of sieving properties of an electrophoretic gel.*

Once the fragments of DNA have been physically separated, the remaining steps in the analysis are directed at an identification of the sizes of the fragments. The size identification steps cannot be done readily if the DNA fragments remain in the separation gel, so they are transferred to a nylon membrane in exactly the same array as they existed in the gel. This transfer is known as Southern blotting and is named for the developer of the technique.

After transfer to the nylon membrane, the fragment locations on the membrane are established. This is done by using a DNA probe. A DNA probe is a short sequence that is complementary to the region of DNA that one wishes to detect. In the case of the C-A-T sequences, the probe must be composed of the letters G-T-A because this letter sequence is the complement of C-A-T. DNA probes can be tagged with radioactivity to facilitate their detection later in the analysis.

The membrane, with its array of DNA fragments, is immersed overnight in a solution that contains the radioactive probe of sequence G-T-A. During this time, the probe molecules will match up with the areas of the DNA fragments that contain the complementary sequence of letters. For the two fragments shown in Figure 3, there will be six probe molecules bound to the large fragment and three probe molecules bound to the smaller fragment. After this procedure takes place, which is called hybridization, the membrane is washed thoroughly to remove any unbound probe.

The final step is directed at determining which areas of the membrane contain radioactivity. The membrane is covered with a sheet of unexposed X-ray film and stored from one to seven days at a very low temperature. During this storage, the radioactive particles expose the X-ray film at the exact location of the DNA fragments that have hybridized with the probe molecules. When the X-ray film is developed, the locations of the fragments that have hybridized with the probe are seen as lines on the film (Figure 4).

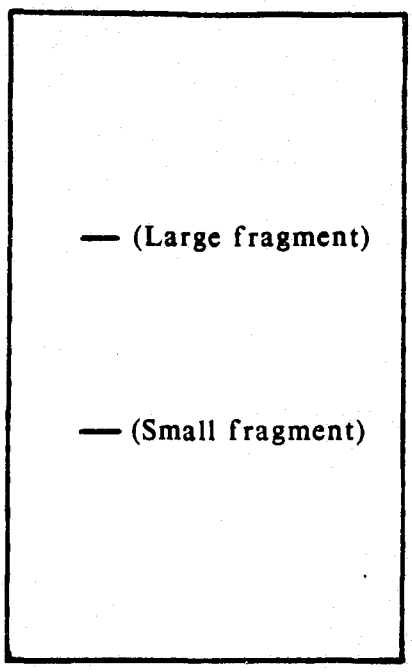


*Figure 4. Appearance of fragments on X-ray film.*

The actual size of each of the fragments is calculated by reference to standards of DNA of known size that are run in parallel with the test specimens. The determination of fragment sizes can be carried out by image analysis techniques.

The fragment pattern shown in Figure 4 is illustrative of the result when a single locus DNA probe is used. Single locus probes are used to detect repetitive sequences that occur at a single location in the DNA from one pair of chromosomes. The maximum number of bands that should be seen on the X-ray film is two (one band is derived from each chromosome). The appearance of more than two bands on the X-ray film suggests that body fluids from more than a single individual were present in the test specimen.

The number of different sized repetitive sequences that occur in the human population that are detected by a given single locus probe is limited, and varies with the DNA probe being used as well as the restriction endonuclease used to cut the DNA strands. One of the DNA probes in use by the FBI Laboratory enables the detection of more than 50 different sized repetitive sequences in the population. Because at most, one individual can possess only two different sized sequences in his/her DNA (when using a given probe), the number of different combinations of size sequences that can be present is large. As the number of combinations becomes large, the chance of two individuals having the same two fragment sizes becomes correspondingly small. Although the use of a solitary single locus probe can reduce considerably the number of potential contributors of a body fluid, additional DNA probes must be applied to the resolved fragments to enable absolute individualization of the specimen source.

To genetically characterize a pair of specimens to the extent that it can be stated that they could have originated from but a single individual, requires the application of several single locus DNA probes, each of which recognizes a different, and genetically unrelated, series of repeated DNA sequences. For example, assume that five different DNA probes will be used to compare the DNA of two specimens. Further assume that the likelihood of two people sharing the same size repeat DNA sequences for each of the probes is 1 in 100. For the five probes, the net likelihood that the two people will match is 1 in 100 X 100 X 100 X 100 X 100 = 1 in 10,000,000,000. Clearly, the use of five such probes would eliminate more potential donors than are living on earth.

Another type of DNA probe that can be used to detect restriction fragments of different sizes is known as a multilocus probe. While similar to single locus DNA probes in that they detect repeated sequences in DNA fragments, they differ in that multilocus probes simultaneously detect DNA fragments from multiple regions of multiple chromosomes. Dr. Alec Jeffreys was the first to recognize the utility of multilocus probes. He coined the term "DNA fingerprinting" to describe the application of multilocus probes to the genetic characterization of forensic specimens.

The procedures for using multilocus probes are essentially the same as for single locus probes. Chromosomal DNA is isolated, cut with a restriction endonuclease, subjected to electrophoresis, and the fragment array is tested with a DNA probe. The band patterns that develop on the X-ray film after using multilocus probes are somewhat

more complicated to interpret than for single locus probes. It would not be uncommon to have more than 20 fragment bands appear on the film. In fact, when using multilocus probes with fragmented DNA, one does not know in advance how many bands will appear. For this reason, it is difficult to recognize a mixture of body fluids from two or more individuals. Because the multilocus probe recognizes sequences from multiple sites simultaneously, the chances of two individuals at random matching at all the band positions is enormously low. It is conceivable that by the use of only a single multilocus probe one would be able to individualize a body fluid specimen.

## STAIN QUALITY/QUANTITY AFFECTS SUCCESS OF RFLP ANALYSIS

Controlled studies in the laboratory have shown that RFLP patterns can be discerned when the analysis was begun with bloodstains as small as 5 uL and semen stains as small as 1 uL (one uL is about 1/50 the volume of one drop from an eye dropper). Because environmental vagaries can have an appreciable effect on body fluid stains, the recovery of DNA from actual case evidence stains will undoubtedly be less than that seen with stains prepared for research studies.

The genetic characterization of forensic biological materials by RFLP analysis requires that the DNA in the cells not be fragmented prior to the initiation of the analysis. Fragmentation of the DNA must be carried out in a controlled manner by digestion with restriction endonucleases. If the chromosomal DNA strands have been broken because the tissue or body fluid stains have been adversely affected by the environment, RFLP analysis may not be possible. A list of potential environmental antagonists of DNA would have to include: sunlight, bacterial growth, heat, moisture, and chemicals. Scientists who perform these procedures will, early in the analysis, conduct a test on the DNA isolated from the stain that measures the extent of DNA deterioration. If this test indicates that extensive deterioration has occurred, the remaining steps in the procedure will not be carried out.

## TYPING OF SUBANALYTICAL QUANTITIES OF DNA

The interchange of biological substances between individuals during criminal activities often involves only trace quantities of materials. Small samples of body fluids and human hair (especially telogen hair) will contain DNA that is qualitatively representative of the donor but is quantitatively inadequate for RFLP analysis. An alternative procedure can be carried out on these subanalytical quantities of DNA to enable the genetic characterization of such specimens; although not to the same extent as is possible by RFLP analysis. This technique is called polymerase chain reaction (PCR). The PCR method repetitively reproduces copies of a sequence of DNA until the quantity of DNA copies is sufficient for testing with a special category of DNA probes.

The mechanism by which PCR effects the duplication of the DNA specimen is composed of three steps. For example, assume that the following sequence of DNA is to be increased in quantity by the PCR procedure.

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

First, the two strands of DNA must be separated by raising the temperature to about 95$^0$C.

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

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

Second, short pieces of laboratory synthesized DNA, called primers, are permitted to hybidize to each DNA strand as the temperature is lowered. The sequence of DNA letters that immediately precede the region that is to be amplified must be known in order to synthesize the primers.

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

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

The third step is called primer elongation and is effected by DNA polymerase and the four letters (A, C, G, T) that compose DNA. The DNA polymerase will start at the end of the primer and, by using the four letters, synthesize complementary copies of each of the two strands.

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

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

After the first round of synthesis has been completed, the temperature of the reaction is once again raised to 95$^0$C to separate the DNA strands. Primers again hybridize with what are now four strands and the temperature reduced so that the DNA polymerase can reinitiate DNA synthesis starting at the primer sites. Each cycle of PCR doubles the quantity of DNA that was present at the beginning of the cycle. Thus, the PCR procedure increases the quantity of DNA strands in a geometric manner (that is, 2, 4, 8, 16, 32, etc). After approximately 20-25 cycles, sufficient DNA should have been produced to enable the testing of the sample with special DNA probes.

PCR generated DNA has been tested with probes that have been produced to hybridize with the regions of DNA that code for the synthesis of cellular transplantation antigens called HLA-DQ. Because the number of HLA-DQ antigens that occur in the population is not large, the degree to which a DNA specimen can be genetically characterized with these probes is considerably less than that achievable by RFLP analysis. However, the typing of PCR-generated DNA with this type of probe is less complicated to carry out than the RFLP technique. Because the differences in the HLA-DQ antigens are due to different arrangements of a fixed number of DNA letters rather than due to different lengths of the same sequence, an electrophoretic separation of DNA is unnecessary. Instead, replicate volumes of PCR-generated DNA are spotted onto a nylon membrane and allowed to dry. The replicate spots are tested with a series of HLA-DQ probes that are individually specific for each of the different HLA-DQ sequences. DNA from one individual will demonstrate binding with a maximum of two different HLA-DQ probes if the person carries different DNA codes for these antigen sequences on each chromosome. If only one probe of the series hybridizes with the DNA, the individual probably carries the same HLA-DQ sequence in the DNA of both chromosomes.

Probes used to test for the HLA-DQ DNA sequences usually are not labeled with radioisotopes as an aid to their detection. The probes can be linked to an enzyme whose catalytic activity produces a color at the location where the probe has been bound.

## DIRECT SEQUENCING OF THE DNA CODE

A third approach by which an individual's DNA can be genetically characterized is by determining the sequence of DNA letters in a region of DNA in which the order of the letters varies greatly among individuals. Such a region appears to be present in the DNA that is found in the energy producing centers of the cell, which are called mitochondria. The sequence of DNA letters in a portion of the double stranded mitochondrial DNA appears to be highly variable, and can potentially serve as a means to genetically characterize the individual source of DNA.

The direct DNA sequencing procedure bears a similarity to PCR in that primers must be attached to the separated DNA strands and DNA polymerase is present to synthesize the complementary strands of DNA. However, the primers are fashioned so that they attach to only one of the two strands and in addition to the four DNA letters that serve as building blocks for the new strands, specially synthesized derivatives of the four letters are present also. Because each of the normal DNA letters is present in the reaction solution as well as the derivative letters, the potential exists for the DNA polymerase to incorporate a derivative letter into the synthesized strand instead of the normal letter. If a derivative letter is inserted, the synthesis of the strand ceases at that point. The termination of the growing DNA strands will occur each time a derivative letter is incorporated. The ratio

of normal letters to derivatized letters is such that the synthesis of some strands will be terminated at each letter position in the strand, while other strands will not be terminated because normal letters have been incorporated.

At the end of the synthetic phase of the procedure, there will be present in the reaction tube, a series of synthesized DNA fragments that differ in length by one letter, with the last letter in each fragment being a derivative.

The next step in the procedure is the electrophoretic separation of the fragments based on size. The fastest moving fragments (shortest) will be those whose synthesis was terminated when the DNA polymerase incorporated the first letter into the strand and did so with a derivative letter. The slowest in electrophoretic migration will be those fragments (longest) that were not terminated until the last letter in the strand was reached and a derivative was incorporated. The sizes of the fragments are determined as the electrophoresis is in progress. As the fragments pass a fixed point in the electrophoretic gel, a scanning device registers fragment passage. In one type of DNA sequencer, each of the derivative DNA letters is labeled with a distinctive fluorescent molecule, enabling the sequencer electronics to determine which derivative letter had been incorporated into that particular fragment. After all the fragments have passed the detector, the complete sequence of letters will have been determined. If necessary, the sequence of the complementary strand can be determined in a similar fashion (using the appropriate primer) as a check on the authenticity of the letter sequence that has been determined in the first strand.

# Expenses Associated With DNA Typing Methods

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**Serology Unit**
**FBI Laboratory**
**Washington, D. C.**

## A. RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

### I. Equipment

The conduct of DNA typing tests through analysis of restriction fragment length polymorphisms (RFLP) does not require any sophisticated items of equipment. Laboratories that currently perform the genetic typing of body fluids by electrophoretic methods probably have a number of the items listed in Table 1. Items in the list marked by an asterisk * would be considered desirable, but not essential, for the conduct of DNA typing.

Other items that are needed such as a pH meter, stirring hotplate(s), balances, glassware, aliquot mixer(s), refrigerator(s) and a source of ice should be in the inventory of every laboratory.

Several types of laboratory hoods should be available in the DNA laboratory. A hood should be dedicated solely to the preparation of radioactively-labeled DNA probes. A fume hood should be available for the handling of organic solvents such as phenol and chloroform. Finally, consideration should be given to the purchase of a laminar flow hood that will protect personnel from biohazards during the initial removal of stains from evidentiary items. The cost of these hoods will range from $3,000 to $6,000 each.

The purified water that is available in the laboratory for solution preparation can be critical to the success of the analysis. At a minimum, water must be passed through a multi-stage ion exchange process followed by distillation, or vice-versa. Assuming that the laboratory is currently using distilled water, the cost of a deionizing system will be approximately $3,000.

Finally, a photographic darkroom is necessary for loading and processing of X-ray film.

### II. Supplies

The typing of DNA by RFLP analysis requires a considerable inventory of consumable chemical reagents and supplies. Some of the biochemical reagents are quite expensive (for example, restriction enzymes and radioisotopes). Other items such as microcentrifuge tubes and micropipette tips, while not expensive individually, are used in large numbers. Based on current rates of utilization in the Forensic Science Research Unit, the supply/reagent costs per specimen analyzed is approximately $12. **This value does not include the cost of the DNA probes.** Presently there is no commercial vendor of the DNA probes that are under consideration for incorporation into the FBI Laboratory DNA typing protocol.

## B. POLYMERASE CHAIN REACTION

While not a DNA typing method in itself, polymerase chain reaction (PCR) is a technique that enables the amplification of subanalytical quantities of DNA to achieve a level that is conducive to DNA typing. The DNA probes used in conjunction with PCR are different from those used in RFLP testing. Probes used with PCR do not enable the individualization of DNA specimens. Moreover, these probes are currently unavailable commercially. Prices will become known when these probes become accessible to the community.

A DNA thermal cycler is needed to carry out the PCR technique. The cost of this instrument is approximately $10,000. Other equipment items are in common with the RFLP technique. Specialized reagent costs for the PCR technique will be about $10 per specimen.

## C. DNA SEQUENCING

A different method that can be used potentially to individualize body fluid specimens entails the direct determination of the sequence of molecules in DNA. While the application of this technique to forensic specimens is not as well-defined as the RFLP technique, it may be incorporated into the forensic laboratory within the next few years. The cost of a DNA sequencer approaches $100,000. Specialized reagent costs for this technique would be about $10 to $15 per specimen.

**Table 1**

| EQUIPMENT ITEM | APPROXIMATE COST/ITEM |
|---|---|
| Autoclave (bench top) | $ 4,600 |
| UV Spectrophotometer | 12,000 |
| UV Transilluminator | 2,300 |
| Electrophoresis power supply | 2,500 (minimum of 2) |
| Electrophoresis tanks | 400 (minimum of 4) |
| Microfuge (general use) | 1,200 |
| Microfuge (dedicated to isotopes) | 1,200 |
| Microliter pipettor (variable range) | 250 (3 required) |
| Water bath | 2,000 (2-3 required) |
| *Vacuum centrifuge | 4,300 |
| Ultra-low temperature freezer | 6,300 |
| *Environmental rotatory shaker | 5,100 |
| *Vacuum oven | 1,000 |
| Gel photography equipment | 1,000 |
| *X-ray film autoprocessor | 7,100 |
| Platform shaker | 1,000 |
| Benchtop radioisotope counter<br>OR<br>Liquid scintillation counter | 2,400<br><br>12,000 |
| Radioactivity survey meter | 250 |
| Intensifying screens (pair) | 300 (5 pair minimum) |

# Radiation Aspects of DNA Analysis

**John P. Riley**
**Gunshot Residue and Metals Analysis Unit**
**FBI Laboratory**
**Washington, D. C.**

Recent advances in DNA technology have provided the molecular tools which afford the forensic scientist the promise of the ultimate discriminating power, the ability to identify a biological specimen as having come from a single individual. The advanced DNA technology involves the extraction of the DNA from the biological specimen, cutting the DNA with restriction enzymes, electrophoretic separation of the cut fragments, transfer of the DNA to a membrane, the reacting of the transferred DNA with radioactive probes and the visualization of the location of the radioactive probes through the use of X-ray film. This developed X-ray film, called an auto-radiogram, provides the pattern which may allow individualization.

Since this process uses probes tagged with radioactive phosphorus 32, various aspects of radiation safety will have to be addressed when considering DNA analysis. Radioactive phosphorus 32 ($^{32}P$) comes under the purview of the Nuclear Regulatory Commission (NRC); therefore, it will be necessary to apply for an NRC Material License. At the time of application, the NRC will require one of the laboratory personnel to have training in radiation safety (from 1 day to a week's training, depending on the type of course attended) and some direct experience with beta-emission sources such as $^{32}P$.

Another major consideration is a "hot laboratory" for processing samples with $^{32}P$. A partial, but not all inclusive list of items needed in this laboratory includes a seamless hood, 1/2-inch plexiglass shielding, safety glasses, beta boxes for storage of $^{32}P$, finger dosimeters, a hot sink and survey meters for the frequent monitoring of the laboratory for accidental spills and contamination. All of these items are available through commercial vendors. If your laboratory is already licensed to perform radioimmunoassay analysis, you may have most of this equipment, and you need only to amend your existing license to include $^{32}P$.

Disposal of liquid waste containing $^{32}P$ must be performed according to Section 20.303 of Title 10, Code of Federal Regulations, part 20, which sets forth the pertinent NRC Rules and Regulations for disposal. Under most conditions, liquid $^{32}P$ waste can be immediately disposed of into the sanitary sewerage, whereas solid $^{32}P$ waste must be allowed to decay approximately 10 half-lives or approximately 5 months prior to disposal. Commercial waste firms are available for radioactive waste disposal, if necessary.

For laboratories with personnel unfamiliar with radioactive materials, the use of a consultant is recommended. Most large universities have radiation safety officers who could serve in this capacity by providing advice on how and where to purchase the $^{32}P$ and assist in obtaining training for personnel, acquiring the NRC materials license, outfitting the laboratory and establishing analytical procedure.

For further information, the following are commercial telephones numbers for the NRC Regional Offices:

Region I - King of Prussia, Pennsylvania
(215) 337-5000
Region II - Atlanta, Georgia
(404) 331-4503
Region III - Glen Ellyn, Illinois
(312) 790-5500
Region IV - Arlington, Texas
(817) 860-8100
Region V - Walnut Creek, California
(415) 943-3700

A forensic scientist commencing study in DNA analysis will benefit from obtaining a theoretical background in the biological and chemical aspects of DNA formation, function, isolation and characterization.

To provide this type of background, the FBI Laboratory in conjunction with the Chemistry Department of the University of Virginia has designed a short course in molecular biology for FBI personnel. The course, entitled "DNA Characterization", will be taught by professors from the chemistry and biology departments of the University of Virginia. Successful completion of the 44 hours of lecture material will earn the student 3 semester hours of graduate credit. The course was designed to present a general overview into molecular biology topics and is not directed specifically toward forensic applications. Its purpose is to provide background material and supplement the theoretical and practical training in the forensic methodologies that are currently under development at the FBI. An outline of the topics to be covered are being listed to assist you in the potential development of similar courses with colleges and universities in your areas.

## SHORT COURSE OUTLINE

Part I.

DNA structure, gene structure, chromosome structure, mitosis, meiosis, classical mapping techniques, human linkage maps, sex linkage.

a. DNA chemistry

Primary structure
Secondary structure
Tertiary structure
DNA denaturation and renaturation
Cot curves
Mismatch and renaturation

b. DNA organization in chromosomes

The nucleus
Nucleolus
Nucleus membrane
Nucleosomes and chromosome condensation

c. Extrachromosexual DNA

Mitochondria
Chloroplasts

d. Mitosis - the accurate copying of genetic information, somatic crossing over
e. Meiosis - independent assortment, crossing over and chiasmata

f. Mendelian genetics

Autosomal markers
Sex linkage
Family trees
Paternity questions

g. Mutations

Point mutations
Rearrangements
Internal crossing over
Natural selection
Polymorphisms (25% of all human loci)

Part II.

a. DNA replication

DNA polymerases
Exonucleases
Initiation, extension, termination
Ligase
DNA polymerization in vitro

b. Transcription

DNA - dependent RNA polymerases
Initiation, elongation, termination
Splicing

c. Translation

The adaptor hypothesis
Ribosomes
Protein synthesis
The genetic code; wobble
Protein polymorphisms

Part III.

DNA typing
DNA extraction and preservation stabilizing

a. Restriction mapping, partial digests, labeling techniques, DNA sequencing, autoradiography, blotting techniques, reprobing

b. Cloning technology

c. Simple and multi-copy polymorphisms
Human versus nonhuman DNA

d. Amplifying specific sequences of DNA with heat-stable DNA polymerase

e. Prospects for technical improvements

f. Linking classical genetic maps with DNA maps

# DNA Bibliography

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The following bibliography of papers and text chapters dealing with DNA analysis is divided into 15 topic areas. Some papers are historical in nature, however, most were recently published. These listings do not represent complete coverage of a topic area but contain selections that should provide sufficient background material necessary for the understanding of a particular subject. The purpose of this bibliography is to provide resource material that is directly related to both the theoretical and practical aspects of the forensic analysis of DNA.

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