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Forensic DNA Technology

Edited by
Mark A. Farley,
James J. Harrington

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FORENSIC DNA TECHNOLOGY

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Preface

The decade of the 1990s promises to be the age of biotechnology. Recent discoveries in this field have resulted, for example, in major advances in understanding disease, in the treatment of environmental pollutants, and in the development of new pharmaceutical products. One of the most exciting applications of this field of science has been the implementation of DNA print technology in the analysis of physiological evidence uncovered during the course of criminal investigations and its related use in the investigation of issues relating to paternity.

DNA, or deoxyribonucleic acid, is the genetic material that provides the code that determines each person's individual characteristics. A number of analytical techniques, previously utilized for a variety of applications by molecular biologists, have recently been utilized to determine the unique characteristics of DNA and thus to permit its use in identifying the source of biological stains such as blood or semen deposited at a crime scene. Since many of these recent discoveries lie at the frontier of biotechnology, however, potential problems in their application to forensic investigation and issues relating to their weight and admissibility in court are only now coming into focus.

Forensic DNA Technology examines both the legal and scientific issues relating to the implementation of DNA print technology in both the crime laboratory and the courtroom. The book has been written for use by nonscientists as well as by those having a degree of technical expertise in the areas covered. Chapters written by a number of the country's leading experts trace the underlying theory and historical development of this technology, as well as the methodology utilized in the restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) techniques. The effect of environmental contaminants on the evidence and the statistical analysis of population genetics data as it relates to the potential of this technology for individualizing the donor of the questioned sample are also addressed. Another chapter deals with proposed guidelines for the use of this technology in the crime laboratory, while still others set forth, from the perspective of the prosecution and the defense, the legal standards for determining the admissibility and weight of such evidence at trial. Finally, the issues of the validation and proposed standards for interpretation of

autoradiograms are brought into focus in a detailed study of actual case work.

In view of the rapidly increasing importance of DNA typing technology, the need for a reliable, up-to-date reference dealing in a single volume with both the legal and the technical issues inherent in this area has prompted the publication of *Forensic DNA Technology*.

About the Editors

Mark A. Farley, Esq. is an attorney with the law firm of Pennie & Edmonds. The firm, with offices in New York City and Washington, D.C., specializes in the protection of intellectual property assets, including patents, trademarks, copyrights, and trade secrets, with a particular emphasis in the field of biotechnology. Mr. Farley was formerly employed from 1977–1984 with the Suffolk County, New York, Division of Medical-Legal Services and Forensic Sciences as Supervisor of the Chemistry Section of the Suffolk County Criminalistics Laboratory. His duties at the laboratory included the chemical and instrumental analysis of pharmaceutical products as well as crime scene investigation and analysis. He has testified as an expert witness in pharmaceutical analysis in over 30 criminal trials.

Mr. Farley is registered to practice before the U.S. Patent and Trademark Office. He holds a Juris Doctor degree (1985) from the Jacob D. Fuchsberg Law Center of the Touro School of Law, where he was a Senior Editor of the Touro Law Review. In addition, he holds a Master of Science degree in Forensic Chemistry (1977) from John Jay College of Criminal Justice of the City University of New York and a Bachelor of Science degree (1974) in Biology from Saint John's University, Jamaica, New York.

He is an active member of the American Chemical Society's Division of Chemistry and the Law, serving on its Executive Committee as Chairman of the Forensic Chemistry Committee. He is a member of the American Bar Association, the New York State Bar Association, the New York Patent, Trademark and Copyright Law Association, and the Northeastern Association of Forensic Scientists.

Mr. Farley has organized and moderated a number of symposia at ACS National Meetings dealing with issues such as the analysis and abuse of steroids, the clandestine manufacture of illicit pharmaceutical products, and the role of the scientist as an expert witness. He served as Cochairman for the Forensic DNA Symposium held during the American Chemical Society's national meeting in Los Angeles in September, 1988.

His professional interests include the protection and licensing of technology and the education of the public concerning the role of the forensic scientist in the analysis of physiological evidence.

James J. Harrington is a Senior Scientist with New Jersey's North

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William C. Thompson, an attorney and psychologist, is an associate

professor in the Program in Social Ecology at the University of California, Irvine. He has published a number of articles on human judgment and decision making and is particularly interested in the ability to draw appropriate conclusions from scientific and statistical data. His interest in forensic DNA analysis arose out of his National Science Foundation funded research on the use of mathematical evidence in criminal trials. In collaboration with molecular biologist Simon Ford, he has published four articles (in addition to the chapter in this volume) on the scientific and legal aspects of genetic identification tests. He served as lead defense counsel during the Frye hearing in *State of New Mexico v. Anderson*, one of the first cases in which the defense raised a serious challenge to the admissibility of the FBI's DNA test. He holds a J.D. from the University of California at Berkeley and a Ph.D. in Psychology from Stanford. Before retiring to the academic world in 1983, he was a civil litigator.

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cases in California in which the prosecution sought to introduce the results of DNA testing and which involved an extensive Frye hearing.

Jan S. Bashinski is the manager of the DNA program of the California Department of Justice, having assumed that post in the fall of 1989 after 25 years as a criminalist and laboratory director with the Oakland Police Department Crime Laboratory. She holds a B.S. degree in chemistry and an M.Crim. in criminalistics, both from the University of California at Berkeley. Throughout her career, her research interests have centered on forensic serology, and she has been project director of two National Institute of Justice sponsored research studies on the genetic typing of sexual assault evidence. Her past professional activities have included service as President of the California Association of Criminalists, Chairman of the Criminalistics Section of the American Academy of Forensic Sciences, and Executive Secretary of the American Society of Crime Laboratory Directors Laboratory Accreditation Board. In her current position with the California Department of Justice, Ms. Bashinski is responsible for establishing a centralized DNA typing laboratory which will analyze DNA evidence as well as creating a DNA offender identification file for the State of California.

Acknowledgments

A book of a scope as wide as that of *Forensic DNA Technology* would obviously be impossible to compile without the advice of numerous legal and scientific experts. Since we relied on so many people, it would be impossible to thank all of them individually. To all of you, we would like to extend our sincere appreciation for your efforts. The editors would, however, like to individually thank a number of people who were especially instrumental in motivating us to finish this work when all appeared hopeless.

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James Harrington: I would like to thank the members of the New Jersey

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The reader is advised that all of the opinions expressed herein are those of the authors and are not necessarily those of the editors, The American Chemical Society, the Division of Chemistry and the Law, Inc., Pennie & Edmonds, or the New Jersey State Police.

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1. *Milestones in the Development of DNA Technology*

J.A. WITKOWSKI, Ph.D.

INTRODUCTION

DNA technology is having an increasing impact on our daily lives. The availability of DNA diagnosis for an ever-increasing number of human inherited disorders has brought significant benefits to families afflicted by these diseases.[1] DNA technology is leading the fight against AIDS[2] and recombinant DNA techniques may result in "tailor-made" drugs and other therapeutic agents.[3,4] However, one of the most spectacular, and certainly one of the most publicized, applications of DNA technology involves so-called DNA fingerprinting.[5,6] The mystique of DNA, together with the apparently infallible identifications that result, is proving to be a potent combination when the evidence analysis is presented in court. It is difficult under these circumstances to realize that DNA typing is the result of a "basic" research project that in itself was based on experiments and theories stretching back over the past 50 years. In this brief introduction DNA typing will be set in its scientific context and some of the milestones in the development of molecular biology will be described.[7]

THE DAWN OF MOLECULAR BIOLOGY

A good year to begin is 1938, the year in which the phrase *molecular biology* was first used, or at least first appeared in print. Warren Weaver, director of the Rockefeller Foundation, used it in his annual report to describe a new field of research, one that was " . . . beginning to uncover many of the secrets concerning the ultimate units of the living cell . . .".[8] It was in large part the Rockefeller Foundation, through the advocacy of Weaver, that nurtured the new field by providing support for the x-ray crystallography of biological molecules.[9] This was a particularly British

science and owed its existence to the strong British tradition of x-ray crystallography developed by W. H. Bragg and his son W. L. Bragg, who won the Nobel Prize for Physics jointly in 1915 (W. L. Bragg was only 25, the youngest person ever to win the Nobel Prize).

1938 was also the year in which Bill Astbury and Florence Bell, at the University of Leeds in England, published the first important x-ray crystallographic study of DNA.[10] Astbury (see Figure 1), a student of W. L. Bragg, had worked on keratins, the major constituents of wool, because Leeds had a large weaving industry. He showed that the α-form of keratin was changed into an elongated β-form when wool was stretched, an impressive demonstration of the power of x-ray crystallography in analyzing the behavior of biological molecules. Astbury was interested in the functional significance of the structures of biological molecules, and he began to analyze all sorts of natural fibers.[11] He and Bell examined a dried film of DNA and concluded that the nucleotides were arranged one above the other at right angles to the fiber axis,[10] (Figure 2). Astbury and Bell were delighted to find that the distance between successive nucleotides in their structure was 3.4 Å, almost identical with the spacing of 3.3 Å between successive amino acids in a polypeptide chain. The experimental results seemed to be clear evidence that there was an interaction between proteins and nucleic acids, the latter acting as a framework for the former. In fact, this correspondence between nucleotide and amino acid spacing was a numerological coincidence and the "pile of pennies" model was wrong.[9,12]

DNA AS THE MOLECULE OF LIFE

Astbury seems to have been interested in DNA simply because it was another natural fiber he could analyze. The first convincing demonstration that DNA did something interesting biologically came in 1944 when Avery, Macleod, and McCarty showed that DNA could act as a carrier of hereditary information.[13,14] Up to that time DNA had been dismissed from such a role because biochemical analyses purported to show that the four nucleotides were present in equimolar amounts, and it was assumed that DNA was a polymer of a simple four-nucleotide repeated unit.[9,15] It was clear that proteins with their 23 amino acids were much more complicated and *a priori* more likely to be the hereditary material. Avery et al. showed otherwise, using the bacterium *Pneumococcus*. *Pneumococcus* type II normally forms smooth colonies when grown on agar, but Avery et al. isolated a variant that formed rough colonies. They were able to transform this rough variant into the smooth form of *Pneumococcus* III using DNA purified from the smooth form of *Pneumococcus* III. DNA alone was able to transfer a genetic character and, in addition, the transformed bacteria remained stable through successive generations. There has since been an interesting debate

Figure 1. W. T. Astbury, the British "bulldog" of x-ray crystallography, who with Florence Bell, made the first detailed analysis of DNA. Reproduced by permission of the Department of Textile Physics, University of Leeds, U.K.

as to whether Avery et al.'s paper was neglected by the scientific community.[14–17] In retrospect Avery's data is convincing evidence that DNA and not protein is the genetic material, but at the time this conclusion was not widely accepted. However, it is clear that this study of Avery et al. is one of the classics of modern biology and one that should have won the Nobel Prize.

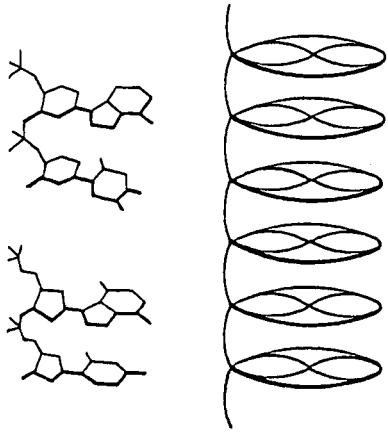

Figure 2. Bell and Astbury's "pile-of-pennies" model for DNA. "Alternative formulae for a pair of (purine and pyrimidine) nucleotides" are shown in the left part of the figure and "the idea is that of a very tall column of discs with a linking rod down one side" (right part of figure). The nucleotides project out at right angles to the axis of the single helix, one above another. Reproduced by permission from *Cold Spring Harbor Symp. Quant. Biol.* 6:109–118, 1938.

DNA AS THE DOUBLE HELIX

This part of the story hardly needs telling, having been the subject of a number of books and a television play.[9,17-19] What does need emphasizing is that it was not a question of luck, although like almost all scientific discoveries, elements of luck were involved. Rather, Watson and Crick, by using a combination of a great deal of shrewd and inspired thinking, together with x-ray crystallographic data from Rosalind Franklin[21] and Maurice Wilkins, derived a structure for DNA that, once seen, had to be right (Figure 3). The crux of the solution was the realization that nucleotides could pair with each other such that an adenine (A) paired with a thymidine (T), and a guanidine (G) paired with a cytidine (C) (Figure 4).[22] Base pairing is the essential characteristic of the DNA molecule that permits all the manipulations of recombinant DNA and DNA typing.

Base pairing was first exploited experimentally in 1960 when it was shown that the two strands of the DNA double helix could be separated and that these separated strands would then hybridize to RNA molecules.[23-25] The importance of this discovery was the demonstration that the base pairing was sufficiently precise that the single DNA strand hybridized specifically with its complementary RNA molecule. Hybridization in solution was used extensively to analyze DNA molecules, but by the mid-1970s a set of procedures, including analysis of DNA fragments by electrophoresis in agarose gels and ethidium bromide staining,[26] Southern blotting,[27] and "nick-translation" to produce radioactive probes,[28] had been developed for hybridization studies. Hybridization in solution is still a major tool in determining the degree of similarity between DNA molecules in studies of gene evolution.[29]

THE ENZYMES

At the same time that studies of RNA, protein synthesis, and the genetic code were going on apace, Arthur Kornberg's laboratory at Washington University, and later at Stanford University, was analyzing the replication of DNA. In 1958, Kornberg reported that he had found a DNA polymerase that required DNA as substrate—a small fragment of DNA to act as a primer—and all four nucleotides.[30] It was now possible to synthesize strands of DNA complementary to another strand.

In 1970, another enzyme essential for recombinant DNA techniques was discovered. Crick had postulated that the only source of genetic information was DNA, and that this information flowed from DNA via RNA to proteins, or from DNA to DNA during cell division.[31] This became known as the Central Dogma, and so the demonstration by Howard Temin and David Baltimore of a retroviral enzyme that reversed this flow of informa-

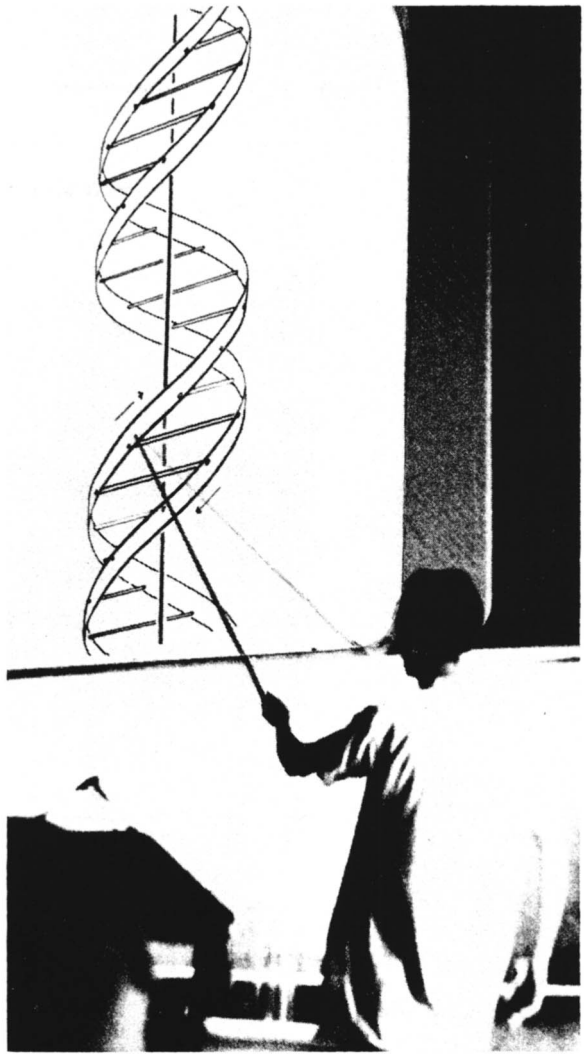

Figure 3. J.D. Watson extolling the virtues of the double helix at the 1953 Cold Spring Harbor Symposium on Quantitative Biology on Viruses. Reproduced by permission of Cold Spring Harbor Laboratory Archive.

tion was unexpected.[32,33] The retroviruses are a group of viruses that have RNA instead of DNA as their genetic information. Temin had suggested that retroviruses had first to make a DNA copy of their RNA genomes, because these viruses multiply within a cell using the cell's DNA-synthesizing enzymes. The enzyme reverse transcriptase does just that, synthesizing

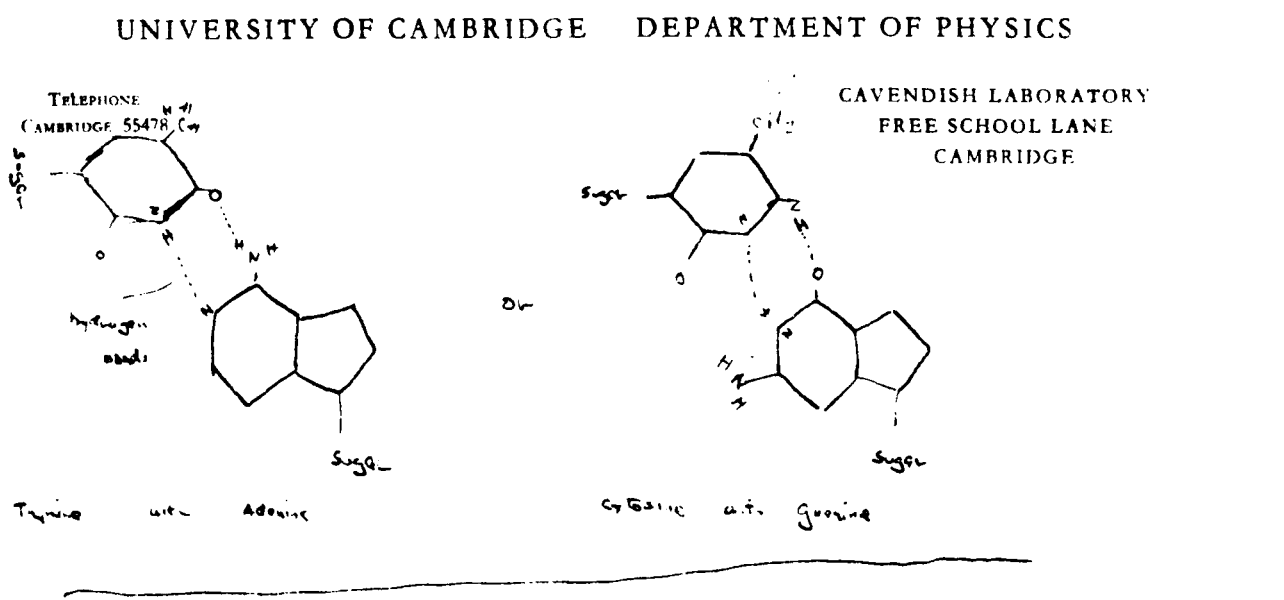

Figure 4. Part of a letter from Watson to Max Delbruck, dated March 12, 1953. The figures are Watson's sketches of the thymidine-adenine (left) and guanidine-cytosine (right) base pairs. Reproduced by permission of Cold Spring Harbor Laboratory Archive.

DNA from an RNA template. The discovery of reverse transcriptase meant that it was possible to take a cell synthesizing a large amount of a specific RNA, for example, globin RNA in a red blood cell, and to make a complementary or cDNA.

In the same year, i.e., 1970, Smith and Wilcox reported their detailed characterization of the restriction endonuclease *Hin*dIII.[34] First described some years before by Werner Arber, restriction enzymes had the curious property of rapidly degrading DNA, unless the DNA came from the same species of bacteria from which the enzyme had been isolated; *Hin*dIII prepared from the bacterium *Hemophilus influenzae* failed to degrade DNA from *Hemophilus influenzae*. Smith and Wilcox showed that *Hin*dIII cut the DNA helix specifically at the sequence:

...A A G C T T...	to give	...A	A G C T T...
...T T C G A A...		...T T C G A	A...

Restriction endonucleases were put to a practical use by Danna and Nathans, who used them to derive a physical map of the genome of the DNA tumor virus SV40.[35] Restriction enzymes are one of the most important tools in the molecular biology workshop. They cut DNA molecules into sizes that can be manipulated, they generate DNA fragments that can be cloned easily, and they are essential for techniques like DNA fingerprinting.

The last of the enzymes that we need to consider is T4 ligase. This enzyme, isolated from the T4 bacteriophage, is able to join together the cohesive ends of DNA molecules that have been cut with a restriction endonuclease.[36] This is exactly the opposite of the actions of restriction enzymes, and the sequential use of restriction enzymes and DNA ligase are critical steps in producing recombinant DNA molecules.

BIRTH OF RECOMBINANT DNA TECHNOLOGY[37]

In the same issue of the journal that contained the paper by Mertz and Davis, there was a paper originating from Paul Berg's laboratory at Stanford University that described the first experimental manipulations of the DNA molecule.[38] Berg's group had used a technique developed by Lobban and Kaiser[39] that added short stretches of nucleotides to the 3′ ends of DNA molecules. Jackson, Symons, and Berg were able to join DNA from simian virus 40 to DNA from the bacteriophage lambda.[38] However, this inactivated the lambda DNA, so that it could not replicate in bacterial cells. (Berg

did not pursue these experiments because of concern about the safety of such experiments; see below.)

The research that led to useful ways of introducing DNA into cells went on in Stanley Cohen's laboratory at Stanford. Plasmids are small circles of self-replicating DNA that occur naturally in bacteria and are responsible for transmitting antibiotic resistance between bacteria. Cohen found a way to introduce plasmids carrying antibiotic resistance genes into *E. coli* cells and to use the antibiotic to select for those bacteria that had taken up the plasmid.[40] Each resistant bacterium gave rise to an antibiotic-resistant clone, with all the bacteria of the clone containing a plasmid derived from the single plasmid in the single bacterial cell that initiated the clone.

One evening, over dinner at a delicatessen at Waikiki Beach, Cohen and Herb Boyer realized how to bring together these various elements—plasmids, restriction enzymes, and other DNA enzymes—for cloning DNA.[37,41] Cohen developed a plasmid called pSC101 that was resistant to the antibiotic tetracycline and contained a single *Eco*RI restriction enzyme site. When pSC101 is cut with *Eco*RI, it is converted into a linear molecule with "sticky" *Eco*RI sequences at each end. They cut the DNA of another plasmid that contained a kanamycin-resistant gene with *Eco*RI, mixed these fragments with the cut plasmid, and joined the fragments with DNA ligase. The DNA was introduced into bacteria, and bacteria that could grow on agar containing both kanamycin and tetracycline were isolated. These bacteria contained replicating recombinant plasmids, formed by the insertion of the kanamycin gene into the pSC101 plasmid. In the jargon of the molecular biologist, the plasmid acted as a vector carrying the cloned DNA into the cell.

The experiments of Cohen and Boyer were not recombinant in the sense that the DNA molecules they created did not combine DNA from different species. This was achieved in 1976 when the first eukaryotic gene, the rabbit β-globin gene, was cloned.[42,43] The next major advance came in 1978, when the enzyme dihydrofolate reductase[44] and proinsulin[45] were expressed in bacterial cells.

The power of cloning comes from three factors. Firstly, it gives us the ability to isolate a single gene or part of a gene from the total human genome of 3×10^9 base pairs. Secondly, once a gene is cloned, very large amounts of the gene can be produced. Each bacterial cell may contain as many as 50 copies of the plasmid with the cloned gene, and all of these are replicated each time the bacterial host cell divides. Thirdly, a gene cloned in a vector can be manipulated in ways that are simply not possible when the gene is part of the long DNA molecule that makes up the chromosome. These manipulations include mutagenesis of specific bases,[46] sequencing,[47,48] and bringing together DNA sequences in novel configurations, for example, in analyzing DNA-binding regulatory proteins.[49]

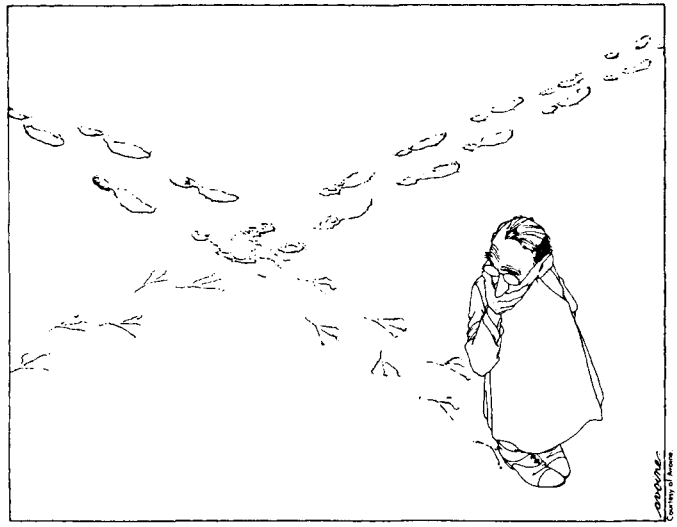

Figure 5. A cartoon by Avoine commenting on the fears of genetic engineering. Reproduced from *The DNA Story*, W.H. Freeman and Company, 1981.

SOCIAL AND POLITICAL RESPONSES[50]

The birth of recombinant DNA technology was not painless (Figure 5). It was realized that there were potential hazards in cloning certain genes into *E. coli*, a bacterial cell that lives in the human intestine. There was also considerable concern that human cancer genes would be cloned when preparing "libraries" of cloned human DNA and that the bacteria carrying these clones would be dangerous. It was not clear how to determine the degrees of hazard involved in such experiments, and a National Academy of Science Committee on Recombinant DNA Molecules was convened to examine these problems. The committee included such luminaries as Paul Berg, David Baltimore, Stanley Cohen, Herbert Boyer, Daniel Nathans, and James Watson. The result of their deliberations was a recommendation that there should be a self-imposed moratorium on certain types of experiments.[51] The first group of experiments involved the construction of plasmids containing genes for antibiotic resistance or bacterial toxins and that might be transferred to bacteria that did not contain those genes. The second group of experiments included those in which DNA fragments from cancer-causing or other animal viruses would be inserted into plasmids. Here the concern was that such DNA fragments that might cause cancer might be transferred through bacteria to human beings and other animals.

This proposal was discussed at a historic meeting of molecular biologists at Asilomar (Figure 6) in 1975,[52] held under the auspices of the National Academy of Sciences, and funded by both the National Institutes of Health

and the National Science Foundation. Then, in 1976, the Department of Health, Education and Welfare introduced very restrictive guidelines governing recombinant DNA experiments.[53] These guidelines caused great consternation in the cloning world, especially amongst scientists working on viruses causing cancer. For example, adenovirus and simian virus 40 can transform normal cells in tissue culture into malignant cells. In 1978, the strict laboratory containment facilities required for using these viruses in recombinant DNA experiments were not yet available in the United States. Workers at Cold Spring Harbor Laboratory went to England to carry out recombinant DNA experiments using the less stringent containment facilities that were required there.[50]

In the meantime, the hazards involved in these experiments have been found to be minimal, except when expressing substances that are known to be dangerous, and the guidelines have thus been progressively relaxed. In fact, research on dangerous pathogens such as the human immunodeficiency virus, the cause of AIDS, has been rendered considerably safer by using recombinant DNA techniques.[2] It is possible, for example, to clone all the various parts of the virus genome and to study these separately from each other so that infectious virus particles are never handled.

Nevertheless, this controversy demonstrated that the uses of recombinant DNA technology were going to be subject to public scrutiny and that scientists could not assume that they would be permitted to do anything that they wanted. This has provided a salutary lesson for forensic applications of DNA techniques and highlights issues of public accountability that need to be addressed.

THE POWER OF RECOMBINANT DNA TECHNOLOGY

In the mid- and late-1960s, scientists like Sydney Brenner and Francis Crick had felt that most of the problems of "classical" molecular biology had been solved. Brenner, in fact, wrote of the need to move on to other problems in biology that were "new, mysterious and exciting."[54] Francis Crick turned first to developmental biology and then to neurobiology,[20] while Sydney Brenner chose to exploit the small nematode worm *C. elegans* as a model system. (This remarkable creature, with a genome of only 8×10^7 base pairs and with the developmental pathways of all its 1000 cells known, is likely to become the multicellular organism of choice for analyzing the molecular control of development.)

The seeming doldrums in molecular biology research were transformed by the ability to manipulate DNA molecules. Recombinant DNA techniques led to a radical revision of the sorts of questions that molecular biologists could ask and hope to answer. This process has continued unabated, with advances in technique leading to advances in knowledge. The methods