ELLIS HORWOOD SERIES IN FORENSIC SCIENCE

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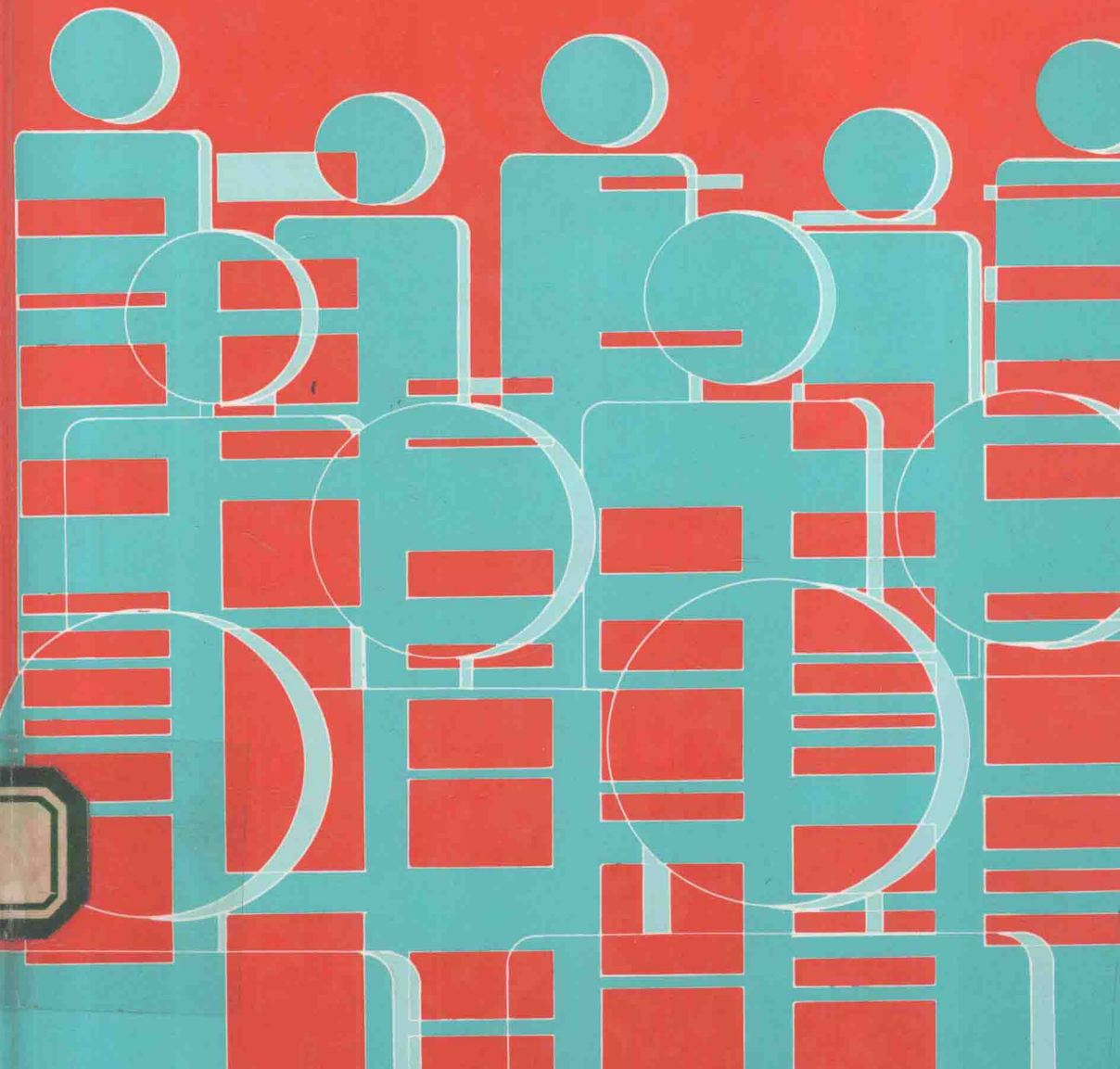
theory, techniques and applications

editors J. Robertson, A. M. Ross, L. A. Burgoyne



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DNA IN FORENSIC SCIENCE
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This up-to-date treatment of DNA in forensic science contains a comprehensive introduction to the underlying science, laying the foundation for a discussion of the technology and methods used, and addressing current applications.

Topics covered include: structure, function and variation in DNA, experimental techniques, hypervariant and intermediate variant probes, a look at DNA analysis in paternity testing, and legal perspectives — amongst others.

By providing the latest information on the uses of DNA in the field of forensic science this book will be of value not only to practitioners but also to all those concerned with the law, and will be of great value to lawyers in coming to terms with this complex field.

Readership: Forensic science practitioners at graduate and post-graduate levels, and legal practitioners.

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Statistics in Forensic Science
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DNA in Forensic Science: Theory, Techniques and Applications
J. Robertson, A. M. Ross & L. A. Burgoyne

Introduction

This is both an amateur's book and a user's book about DNA with the focus being on the practically useful information that can be gained from DNA in the forensic context. However, as the basic biology of DNA is inextricably bound up with the technology of gaining that information and its interpretation, these fundamentals are also covered.

Here is a very considerable challenge, a place where the legal practitioner and the scientific expert *must* meet. There is no choice; neither alone suffices. We hope that these chapters will help in this meeting.

For the lawyer, the uninformative acronym 'DNA' is not an encouragement to read on, and worse, its full name, deoxyribonucleic acid, not only confirms this bad initial impression but also, dangerously, assures one that this is the very sort of substance that is best left to the ivory towers of academia. Nothing could be further from the truth; DNA is fast becoming one more of those familiar modern monsters, another of those bodies of irresistible new knowledge that inevitably savages our old ethical landscape, compensates us with novel solutions to old problems but then leaves us a whole set of new problems.

The intent of this book is to allow the reader to access this complex field as little or as much as they wish. Thus some chapters are clearly more for the expert than others whilst there are chapters which are squarely aimed at the legal practitioner.

The first two chapters deal with the basic structure and organization of DNA in the organism. This lays the foundation for an understanding of the basis for individualization within a population. DNA is a relatively simple structure but like many things legal and scientific the simplicity is often lost in a mixture of jargon and endless details. We hope that these chapters in conjunction with the glossary help to clear away any confusion.

Those of us who were biology students twenty years or more ago really appreciated the enormous bank of diversity within the human genome. Chapter 1 in particular leads the reader step by step into the structure of DNA revealing how this diversity is encompassed in such an apparently simple structure.

Molecular biology has undergone an explosive development phase in the last few

years and, inevitably, in such a rapidly moving field, current knowledge will date. Indeed some details in this book will be superseded even before it reaches the shelf! However the basic material presented in the first two chapters should not alter beyond recognition in the foreseeable future.

In some ways this will be less true of the information presented in the middle of the book. Here, our authors have attempted to cover some of the currently useful protocols and approaches used for forensic applications. At present, there are three broad approaches in application or development. The earliest to be successful, exemplified by the so-called 'minisatellite' technology of Jeffries and co-workers, then the less sensitive, but simpler, 'local detail' approach using 'single loci' and now the highly sensitive PCR (polymerase chain amplification) approach which promises to have the interpretive simplicity of the second approach but with the ability to work with ultra-low amounts of evidential material.

The non-scientist may well wonder why one of these approaches would be selected over the others and what are the underlying principles behind the methods which will remain long after any particular recipe has been superseded.

The main lesson is that all the current technical options merely represent the different prices to be paid in accessing different aspects of the large store of information within the DNA of a human being and the paying of these prices, that is to say, the choosing of a technology, can only be done wisely when one has carefully weighed a number of practical factors. The body of available technology may change its details but the necessity of weighing these factors is most unlikely to change.

This problem of how much to pay for the information begins with the basic questions.

How much biological sample do you have access to?

How much has it been subjected to the random degradative processes of decay?

What amount of information are you prepared to pay for?

And what background of knowledge exists for the interpretation of the information that each method extracts?

How do these questions lead to a choice of technologies?

The current names of the technologies may be of little lasting interest as this is a field of fashion in names and acronyms like 'minisatellites', 'macrosatellites', RFLP, PCR and VNTR and these collections of letters will probably change as technical improvements accumulate and transform. However, it does seem that there are two broad categories within these technologies, there are the 'broad brush' technologies for extracting information from DNA and there are those that concentrate on local details. The 'broad brush' methods are those that have a quick look at some of the grosser features of the arrangement of the genetic information within DNA. These methods usually require high-quality DNA and large amounts of DNA and are thus only really well adapted to paternity studies and clinical usage. Their advantage is speed and convenience and, currently, in the accumulated experience of their interpretation. The broad brush approaches do differ individually from each other, some will tolerate a little more degradation of the DNA than others, some require less DNA than others, some give less 'gel bands' per track and thus less confusing results than others. Then again, these latter require more 'tracks' and this means even more tissue and more work-cost. The 'minisatellites', the VNTR, and the

'macrosatellites' are 'broad brush' methods perfectly *valid* for criminal studies but requiring so much high-quality DNA that they are not commonly applicable.

Alternatively, the methodology that concentrates on local detail, selects out a small section of the DNA information and interprets it in detail. Currently there are two technologies that fall into this category. Firstly there is the old technology of 'unique sequence RFLP' often only referred to as 'RFLP'. This requires much high-quality DNA and produces only a little information per unit effort. It is thus only highly suitable for clinical purposes and is of some value in paternity studies. Once again, it is valid for criminal detection but unlikely to be usable.

Then there is the PCR (polymerase chain reaction) technique and the allied processes. This technique amplifies even the most minute traces of DNA from tiny and degraded tissue samples. A combination of this technique with 'amplifer sequencing' promises to be highly applicable to criminal science. Its current disadvantage is its newness and the caution required in the interpretation of its results. However, there can be little doubt that this or derivatives of it, will be the main basis of criminal detection technology in the future.

To the scientist the choice of technology will depend on the type of material to be analysed. The broad-brush approaches are most suited to parentage testing where relatively unlimited, undegraded sample is available. For blood stains and other 'criminal' case-samples PCR is likely to be the method of choice in the future. Also, and regrettably, it is also becoming clear that the choice for any particular laboratory may be dictated less by scientific criteria than by commercial pressures.

Whatever the method applied there are now clear warning signs to the forensic community that the introduction of DNA into the courts will not be without challenge and problems. It is the responsibility of the scientist to proceed with caution and to make certain that exemplary standards of quality assurance are met. Recent evidence would suggest that in our haste to apply DNA technology these standards have not always been defined much less agreed by the forensic community at large!

Our final chapters present a lawyer's and scientist's perspective of the problems which DNA may encounter as it reaches the courts.

The challenge in assembling a volume like this together has been considerable. Chapters have been included on topics which were not even conceived of when the book was begun. The temptation to wait until the field has matured infects everyone but DNA is in use *now* and so the field must be examined now, and by all of those concerned. The scientist and the lawyer must both accept the challenge of the new technology and we hope that this volume will provide considerable help to both parties.

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1

Structure and function of DNA: an introduction

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1.1 INTRODUCTION

We commence life as a single celled **zygote**, a fertilized egg about 0.1 mm in diameter and weighing less than 1 μg. Replication of this cell, and subsequent differentiation, give rise to a large diversity of specialized tissues, culminating in an incredibly complex yet exquisitely coordinated human adult consisting of more than 10^{14} cells and weighing about 70 kg. The bulk of the cells in such an organism (the **somatic cells**) are mortal and do not directly contribute to future generations. In a sense, immortality is achieved by transmission of genetic material to future generations *via* successful reproduction. The reproductive cells (**gametes**) retain the potential, when combined with a gamete supplied by the opposite sex, for repeating the whole course of development from zygote to adult. The numerous biological 'instructions' that enable these developmental and functional processes to occur are encoded in the molecular configuration of the chemical of heredity, **deoxyribonucleic acid (DNA)** which, together with **protein**, constitute the 23 pairs of **chromosomes** in each somatic cell. Some DNA also occurs in the **mitochondria**, cellular organelles concerned with the production and chemical storage of energy. Thus it is to the chromosomal and mitochondrial DNA molecules that we must look for answers to what are probably amongst the most fascinating questions about life: how is biological information encoded in DNA, how is it transmitted from cell to cell and from generation to generation, and how is the expression of this information regulated in time and space? The more modest aims of forensic science, however, are concerned with developing reliable methods for establishing identity based on naturally occurring differences between individuals. Therefore, questions of direct relevance to forensic science are: what genetic differences exist between the DNA of an individual and between the individuals of a population, and how can these differences be utilized to advantage in forensic analyses?

The recent widespread application of the so called 'genetic engineering' techniques, which enable DNA to be manipulated and its structure explored with a high level of precision, have brought about a 'revolution' in the biological sciences which will, in the course of time, pervade many aspects of human endeavour. It will become desirable, and in many instances essential, particularly for those of us who completed our formal education before the advent of the DNA revolution, to gain a basic understanding of the structure and function of DNA and the principles and techniques involved in its manipulation. This is especially so in the field of forensic science where the pressures for applying new techniques are strong, and where application of the new DNA technology promises so much. Fortunately, despite the seemingly complex biological role of DNA, its structure and properties relevant to its use in forensic science can be appreciated without recourse to more than an elementary understanding of molecular genetics and biochemistry. The purpose of this chapter is to outline the general properties of DNA, with emphasis on its structure and function, in order to provide a framework against which the more specialized areas covered in the remainder of this book can be evaluated.

1.2 DNA AS THE CHEMICAL OF HEREDITY

In the early 1900s it became clear from studies on chromosome behaviour during cell division, and especially from studies of **meiosis** (the type of cell division that gives rise to gametes), that **genes**, the theoretical (and at the time somewhat mystical) units that obey Mendel's laws of inheritance, could be given a physical reality by supposing that they were an integral component of chromosomes. These are the thread-like structures that could be observed microscopically in the nucleus during cell division. The challenge then shifted towards giving genes a chemical reality. Chromosomes were known, at least since the early 1920s, to be composed of DNA (see 1.3) and protein (see 1.8) so these two substances became the main contenders for the chemical constituent of heredity. For many years, in fact up until at least 1950, protein was the front runner, partly because it was known to occur in a wide variety of forms suggesting a potential complexity capable of information storage. DNA, on the other hand, was thought at this time to have a simple homogeneous structure quite incapable of carrying information encoded in its molecular configuration. Chargaff (1950) showed that the earlier ideas of DNA homogeneity were incorrect, and that DNA existed in a wide variety of chemical configurations. His findings, when coupled with some earlier and very elegant genetic experiments using micro-organisms (see Hershey & Chase 1952) convinced biologists that DNA was indeed the genetic material. This conclusion was ultimately consolidated by the exciting discovery in 1953 of the structure of DNA by James Watson and Francis Crick (Watson & Crick 1953), an event which marks the birth of modern molecular genetics. The Watson and Crick structure of DNA, based on X-ray diffraction studies and molecular model building, explained for the first time not only how this molecule could encode biological information, but also how the information could be precisely replicated during cell division.

A convenient feature of DNA particularly when compared with many other biochemical macromolecules, including proteins and **carbohydrates**, is the basic simplicity of its assembly.

1.3 THE STRUCTURE OF DNA

The structural units of DNA are deoxyribonucleotides, **nucleotides** for short. Nucleotides consist of three components:

(1) a 5 carbon sugar, deoxyribose;
(2) one or more phosphate groups (the nucleotide residues in DNA have a single phosphate group but the precursor molecules for DNA synthesis are nucleotide triphosphates), and
(3) a nitrogenous **base**.

The four different DNA nucleotides (listed below) possess identical sugar and phosphate groups but different bases.

Nucleotide	*Corresponding base*	
deoxyadenosine monophosphate (dAMP)	adenine	(A)
deoxyguanosine monophosphate (dGMP)	guanine	(G)
deoxycytidine monophosphate (dCMP)	cytosine	(C)
deoxythymidine monophosphate (dTMP)	thymine	(T)

The structure of the four bases, and the structure of a DNA nucleotide, are shown in Fig. 1.1.

A molecule of DNA is composed of the four nucleotide sub-units linked to form a long unbranched polynucleotide chain (Fig. 1.2). The nucleotides are linked by covalent phospho–diester bonds joining the 5′ C atom of one sugar to the 3′ C of the adjacent sugar *via* a phosphate group. Hence the 'backbone' of the DNA molecule is an invariant alternating series of sugar and phosphate residues. The specificity of DNA resides in its **base sequence**. (The terms 'base sequence', 'DNA sequence', and 'nucleotide sequence' are often used interchangeably). Note that the terminal nucleotides of single stranded DNA molecules have either a free 5′ phosphate or a free 3′ hydroxyl group (Fig. 1.2). This feature is used to orient DNA molecules. Cellular DNA occurs predominantly in a double stranded (duplex) form, the two polynucleotide strands being coiled around a common imaginary axis to form a double helix with bases projecting into the 'inside' of the helix, their planes being perpendicular to the helical axis. The two strands are linked by **hydrogen bonds** between pairs of bases, so called **complementary base pairs** (Fig. 1.1). Because of steric constraints imposed by the dimensions of the helix and atomic structure of the bases, adenine on one strand always pairs with thymine on the other *via* two hydrogen bonds, and guanine pairs with cytosine via three hydrogen bonds. These base pairing rules (A-T; G-C) are absolutely fundamental not only to the structure and function of DNA and **ribonucleic acid (RNA)** (see 1.4), but to many other biological processes not dealt with in this book.

The two strands of a duplex DNA molecule have opposite polarity and are said to be 'anti-parallel'; they are not identical in sequence. However, the base pairing constraints referred to above ensure that the sequence in one strand is the complement of the other. Therefore to specify the information content of a given duplex DNA molecule, it is necessary to record the base sequence of only one strand. By