

DNA - an analytical chemist's view

DNA-based procedures are becoming increasingly common within the analytical laboratory. This is especially the case for food analysis where DNA-based methods are often the procedures of choice for identifying and quantifying genetically-modified organisms and food authenticity analysis. Although these methods are often classed as being in the realm of molecular biology the underlying principles involved definitely lie within the expertise of the chemist.

It is considered important therefore that a series of AMC Technical Briefs are developed to foster good practice in this complex analytical area. This Technical Brief is designed to be a general introduction to DNA and will be followed by others dealing with the basic principles of DNA-based analytical procedures, the potential pitfalls when interpreting analytical data derived from such methods and other DNA-based analytical issues.

DNA

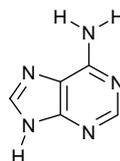
DNA (deoxyribonucleic acid) is found in virtually every plant, micro-organism and animal cell and carries genetic information from one generation to the next. Segments of DNA that carry genetic information are called 'genes'. Genes determine the nature of the cell, control cell growth and division, and direct the biosynthesis of enzymes and other proteins required for cellular function. In cells DNA is organised into structures called chromosomes. The information encoded in DNA is translated into protein via another nucleic acid, ribonucleic acid (RNA).

In 1953 Francis Crick and James Watson published their seminal work on the structure of DNA, deduced from results from previous studies and X-ray diffraction data from Maurice Wilkins and Rosalind Franklin. They built a model that incorporated all known features of DNA, and proposed the now famous double helix structure which is commonly referred to as the Watson-Crick Model. In 1962 Crick, Watson and Maurice Wilkins were awarded a Nobel Prize for this work, in which Rosalind Franklin might have been expected to share. However the award is not made posthumously and by 1958 Rosalind Franklin's short but productive life was over.

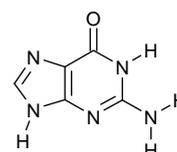
Nucleotides

Just as proteins are polymers made of amino acids, nucleic acids (such as DNA and RNA) are polymers made from individual building blocks called

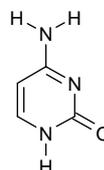
nucleotides that are linked together to form long chains. Nucleotides are composed of three chemical moieties: a heterocyclic amine base, a phosphate group and a pentose sugar (deoxyribose in DNA and ribose in RNA). There are four different heterocyclic amine bases in deoxyribonucleotides. Two are substituted purines (adenine and guanine), and two substituted pyrimidines (cytosine and thymine). Adenine, guanine and cytosine also occur in RNA, but thymine is replaced in RNA by a different pyrimidine called uracil.



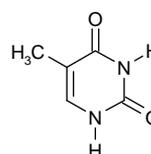
Adenine



Guanine



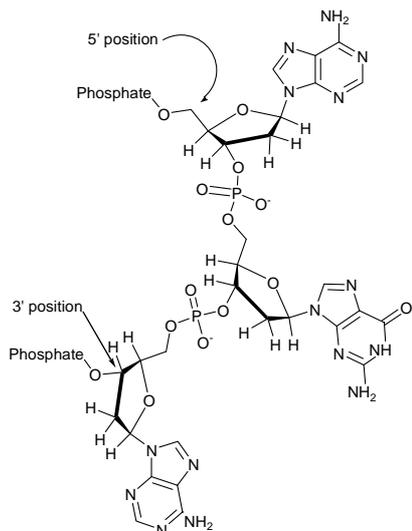
Cytosine



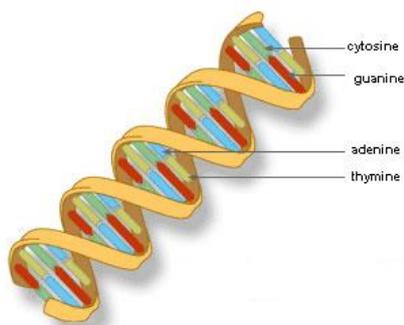
Thymine

Nucleotides join together in DNA by forming a phosphate ester bond between the 5'-phosphate component of one nucleotide and the 3'-hydroxyl on the sugar component of another nucleotide. These asymmetric bonds mean that the DNA strand has a direction and as a consequence one end of the nucleic acid polymer has a free hydroxyl at C3' (called the 3' end) and the other end has a phosphate residue at C5' (the 5' end).

The identity of a gene and the function it performs are determined by the number of nucleotides and the particular order in which they are strung together; this is known as the 'sequence' of the gene. The sequence of nucleotides in a chain is described by starting at the 5' end and identifying the bases in order of occurrence. Nucleotides are named after the heterocyclic base they contain and rather than write the full name of each the general convention is to use simple abbreviations – A for adenine, T for thymine, etc. Thus a typical DNA sequence might be written as –T-A-G-G-C-T-.

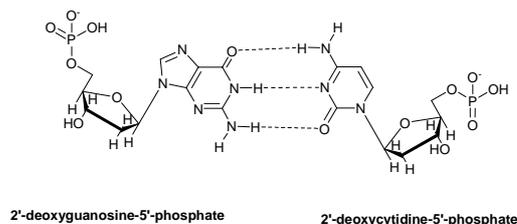
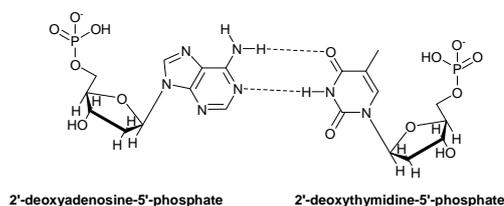


According to the Watson-Crick model, DNA consists of two polynucleotide strands coiled around each other in a double-helix.



(Image supplied by the Food Standards Agency)

The two strands run in opposite directions and are held together by hydrogen bonds between specific pairs of bases. A and T form two hydrogen bonds to each other but not to C or G. Similarly, G and C form three hydrogen bonds to each other but not A or T.



The two strands of the DNA in the double helix are not identical; they're complementary. Whenever a C base occurs in one strand, a G base occurs opposite it in the other strand: similarly for A and T.

This complementary pairing of bases explains why A and T, and C and G, are always found in equal amounts in DNA.

For a biological molecule DNA is unusually stable. DNA carries genetic information/code needed to construct and operate an organism. This code is based on the order of nucleotide triplets (GAA, CTG etc.) in a gene which specify the order of particular amino acids in a protein. Other sections of DNA are responsible for switching genes on and off and regulating how much of each type of protein is made. A detailed discussion of DNA/RNA function and the fundamental processes of replication, transcription and translation can be found in any good textbook on molecular biology. However, the Watson-Crick model provides a mechanism by which DNA molecules are able to reproduce into exact copies of themselves (e.g. during mitosis). This is an enzyme-catalysed process that begins with a partial unwinding of the double helix. As strands separate and bases are exposed, new nucleotides line up on each strand in an exactly complementary manner, A to T, C to G and so on. Enzymes catalyse the production of the phosphate ester bond between the complementary bases and two new strands begin to grow. Each new strand is complementary to its old template strand, and two new identical DNA double helices are produced.

The inherent stability of DNA and its ability to act as its own template for replication forms the basis of many DNA-based analytical procedures that will be discussed and described in further Technical Briefs in this series (e.g. the polymerase chain reaction and the use of DNA-based techniques in food analysis).

Further reading

- *A Structure for Deoxyribose Nucleic Acid (1953)*
The seminal Watson and Crick letter to Nature in April 1953.
(www.nature.com/nature/dna50/archive.html)
- *The Discovery of the Molecular Structure of DNA – The Double helix (2008)*
(http://nobelprize.org/educational_games/medicine/dna_double_helix/readmore.html)
- B. Alberts, A. Johnson, P. Walter, J. Lewis, M. Raff, and K. Roberts "Molecular Biology of the Cell" Garland Publishing Inc, US; 5th Reference. Ed edition (31 Dec 2007); ISBN-10: 0815341113

This Technical Brief was prepared for the Analytical Methods Committee by Dr A P Damant.

<p>CPA Certification I certify that I have studied this document as a contribution to Continuing Professional Development.</p> <p>Name.....</p> <p>Signature.....Date.....</p> <p>.....</p> <p>Name of supervisor.....</p> <p>Signature.....Date.....</p>
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