In other words there are 4.0 x10^5 phosphodiester groups in the basic form to one in the acidic form at pH 7.0.

There are a number of shorthand abbreviations a linear polymer of deoxyribonucleotides. One abbreviation is-

The above can be further abbreviated as

By convention the 5' end of a polynucleotide is placed to the left in such representations.

Since the covalent binding of nucleotides fixes the sequence of bases in the polymer, the following is often used to describe the sequence-

The sequence of bases from the 5' end to the 3' end is referred to as the primary structure of a nucleic acid.

The significance of the sequence of nucleotide bases is that it encodes the sequence of amino acids in a protein.

A sequence of three nucleotides provides a code word (codon) for an amino acid.

In the second semester you will consider how the information in DNA is transferred to mRNA as a complementary sequence of nucleotide bases in a process called transcription, and then how the sequence of bases in mRNA is translated into a sequence of amino acids.

The relationship between the base sequence in DNA, the nucleotide sequence in mRNA, and the amino acid sequence in proteins is indicated in Fig 5.18.

Fig 29-3 gives the standard genetic code, i.e., the triplet codons in mRNA that specify a particular amino acid.

U refers to the nucleotide base uracil that corresponds to thymine in DNA.

The bases in the first column correspond to the first base of the codon, the next four columns to the second base of the codon and the last column to the third base of the codon.

Thus GGG codes for the amino acid Gly (glycine). Trp (tryptophan) is specified by the codon UGG.

Note that for many amino acids there is more than one codon, i.e., UAU and UAC specify the amino acid Tyr (tyrosine).

A few codons are used as start and stop signals.

Two methods have been widely used to determine the sequence of nucleotide bases in DNA.

One is referred to as the chemical cleavage method developed by Maxam and Gilbert. The second is referred to as the chain-terminator or dideoxy method developed by Sanger.

Both methods depend on starting with a homogeneous sample of DNA, i.e., many molecules corresponding to one segment of the extended DNA molecule.

This segment is usually prepared by treating isolated DNA with an enzyme, a protein catalyst, called an endonuclease (restriction enzyme) that specifically hydrolyzes DNA at a particular recognition sequence.

For example, the enzyme EcoR1 recognizes the sequence 5' GAATTC 3' and hydrolyzes the phosphodiester bond between G and A nucleotides.

The recognition sequence of other such restriction enzymes are shown in Table 28-5.

Treatment of DNA with such an enzyme will result in a finite number of fragments corresponding to hydrolysis at each of the recognition sites.

Consider the fragments that would result from treatment of the following DNA molecule of 67 nucleotides represented as a single chain-

1 10 20 30
AGCTCGACC**GAATTC**GTCAAGCGCGTC**GAATTC**
40 50 60 67
CTAGTGGCCCAGTCGAGCTCCGGACTT**GAATTC**G

↓EcoR1

AGCTCGACC**G**
AATTCGTCAAGCGCGTCG
AATTCCTAGTGGCCCAGTCGAGCTCCGGACTTG
AATTCG

Since most DNA molecules have two complementary strands, treatment with EcoR1 cleaves both strands giving fragments with unpaired nucleotides at each end, as shown in the handout.

The unpaired nucleotides are referred to as sticky ends because they can pair (H-bond) to the sticky ends of other DNA molecules.

While the number of fragments is finite, for an average human DNA molecule of 125 million nucleotides in length, thousands of fragments of different length result from the treatment with a restriction enzyme.

It is not technically possible to separate the fragments from each other with the purity required for sequencing.

Each of the DNA fragments in the mixture may be inserted into a DNA of known sequence, such as a plasmid (circular DNA).

The plasmid DNA is first treated with the same restriction enzyme so that the sticky ends of the cleaved plasmid DNA match those of the unknown fragments as shown in Figs. 25B.1 and 2.

Ligation (formation of phosphodiester bonds) of each of the fragments to a cleaved plasmid molecule results in the formation of recombinant DNA molecules as schematically represented below

The resulting mixture of so-called recombinant DNA molecules can be added to a suspension of bacteria such as Ecoli. The bacteria can take up the recombinant DNA molecules from the solution. Usually a single bacterium takes up only one DNA molecule.

When the bacterial cell suspension is spread on an agar nutrient plate and allowed to grow, individual colonies will form corresponding to a single cell that has multiplied with each cell containing many replicated copies of one recombinant molecule.

Return