Using molecular marker technology in studies on plant genetic diversity

DNA-based technologies

DNA basics

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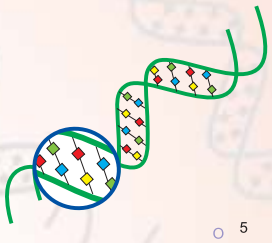
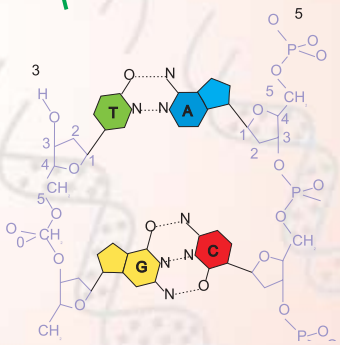
The DNA molecule: structure and features

DNA and RNA are molecules made up of strings of nucleotides



A nucleotide consists of:

- A pentose sugar
- A phosphate group
- A nitrogenous base



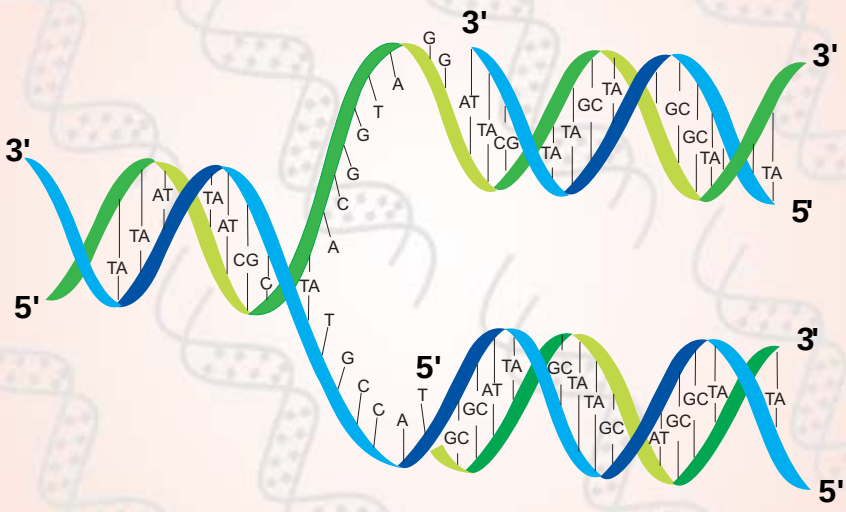
The building blocks of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are nucleotides. A nucleotide consists of:

- A pentose sugar; in DNA, it is deoxyribose, and, in RNA, it is ribose
- A phosphate group
- A nitrogenous base, which can be a:
  - Purine base—adenine (A), guanine (G).
  - Pyrimidine base—cytosine (C), thymine (T). In RNA, uracil (U) replaces thymine

The DNA molecule comprises a chain built from four simple building blocks (A, G, C and T) that are assembled to form a double helix. The helix consists of two strands, each with a sugar-phosphate backbone, held together by a weak hydrogen bond between the bases adenine-thymine (two hydrogen bonds) and cytosine-guanine (three hydrogen bonds).

The shapes of A and T, and of C and G are 'complementary' and form the reason why DNA may copy itself. Two chains of backbones and bases running in opposite directions (antiparallel) form the double helical structure. The order or 'sequence' of these bases along the chain forms the genetic code that carries the precise genetic instructions for the organism to function.

The DNA molecule: replication

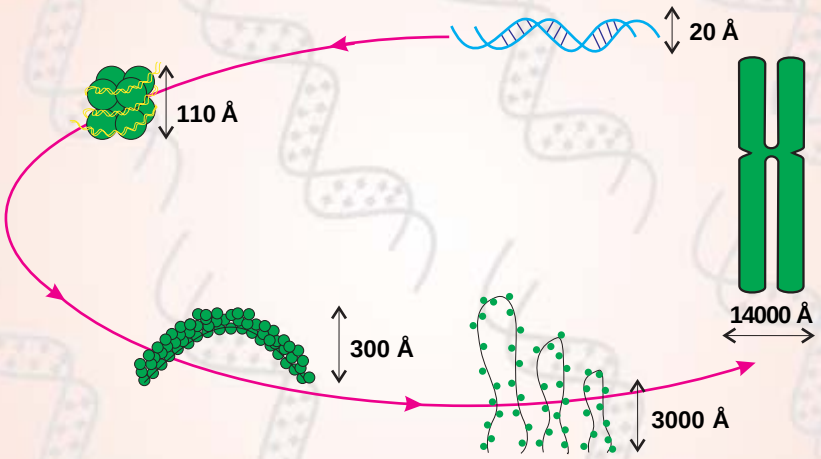


Adapted from Griffiths *et al.* 1996

Under certain circumstances (i.e. during cellular DNA replication), the two chains of the DNA molecule separate. The RNA polymerase synthesizes a short stretch of RNA complementary to one of the DNA strands at a particular site, known as the replication start site.

This short section of RNA acts as a primer for the DNA replication to start. New DNA bases come in at the 3' end and adhere to their complementary pair on the template DNA strand. The new bases are then adjoined to make a 'daughter' DNA chain. As nucleotides are always added at the 3' end, DNA synthesis occurs from a 5' to 3' direction. This process occurs for each of the original chains of the parent DNA molecule.

The DNA molecule: squeezing into chromosome



Å = angstrom, a unit of length that equals to $1/10^{-9}$ m

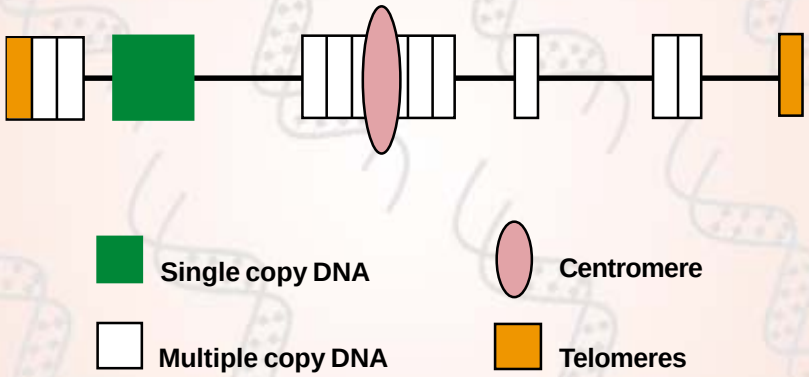
A DNA molecule is much longer than a chromosome, so a mechanism is needed to densely fold and pack the DNA fibre.

The mixture of material of which chromosomes are formed is called chromatin, and is the sum of the DNA molecule plus some proteins. In eukaryotes, DNA is condensed with histone and non-histone proteins, and some RNA. Histones are organized into nucleosomes and give the coiled DNA a bead-necklace appearance. Additional coiling of nucleosomes results in a solenoid conformation, with another level of packaging necessary to arrive at the chromosome structure.

Chromosomes are made up of euchromatic regions, lightly packed and containing most of the active genes, and heterochromatic regions, densely packed and apparently inactivating genes by surrounding them. Heterochromatin is often found around centromeres.

The definition of angstrom is modified from Merriam-Webster Online (http://www.m-w.com)

The DNA molecule: sequence organization



Adapted from Flavell and Moore (1996)

Eukaryotic DNA may be grouped in different types or classes:

- Single-copy, protein-coding genes

- DNA present in multiple copies:
  - Sequences with known function
    - Coding
    - Non-coding
  - Sequences with unknown function
    - Repeats (dispersed or in tandem)
    - Transposons

- Spacer DNA

  Numerous repeats can be found in spacer DNA. They consist of the same sequence found at many locations, especially at centromeres and telomeres. Repeats vary in size, number and distribution throughout the genome, making them highly suitable for consideration as molecular markers.

Reference:

Flavell, R.B. and G. Moore. 1996. Plant genome constituents and their organization. *in* Plant Genome Isolation: Principles and Practice (Foster, G.D. and D. Twell, eds.). John Wiley & Sons, Chichester, NY.

The DNA molecule: cytoplasmatic DNA

- Cytoplasmic DNA
  - Chloroplast DNA (cpDNA)
  - Mitochondrial DNA (mtDNA)
- Features:
  - Maternal inheritance
  - Differing rates of evolution (gene order vs. nucleotide sequence)
  - Slow accumulation of mutations

Smaller amounts of DNA are found in the cytoplasm outside the nucleus—in the chloroplasts (cpDNA) and mitochondria (mtDNA). Chloroplasts and mitochondria each have their own unique 'chromosome', with several copies. These genes also code for their own translation and transcription of organellar components, and play highly specialized roles in the expression of the phenotype of the organism to which they belong.

Organellar DNA is commonly, but not always, inherited only through the maternal parent, a pattern known as maternal inheritance.

The DNA sequences of cpDNA and mtDNA have their own peculiarities. Plant mtDNA appears to evolve rapidly with respect to gene order, but slowly in nucleotide sequence. Why the accumulation of mutations is slow is not properly understood, but may be a result of the presence of either a highly efficient DNA damage repair mechanism or a relatively error-free DNA replication system. Conversely, the rate of cpDNA evolution usually appears slow, in terms of both primary nucleotide sequence and gene rearrangement.

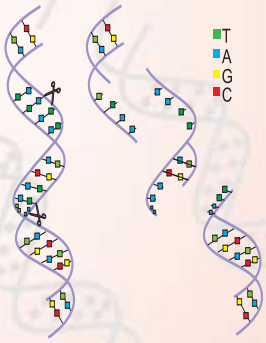
DNA technology

DNA technology involves the concepts of:

- Restriction enzymes
- Nucleic acid electrophoresis
- DNA polymorphism

Restriction enzymes

- Each restriction enzyme cuts the DNA into defined fragments by acting at specific target sequences
- They form either sticky or blunt ends
- Types of restriction enzymes:
  - Types I and III cut double-stranded DNA outside the target sequence
  - Type II identify 4, 5 or 6 bp sequences and cuts inside the sequence



Bacteria produce restriction enzymes as a defence mechanism against bacteriophages. These enzymes belong to a class that cleave (or cut) DNA at specific and unique internal locations along its length. As a consequence, they are also called endonucleases. These enzymes act as scissors, cutting the DNA of the phages and inactivating them.

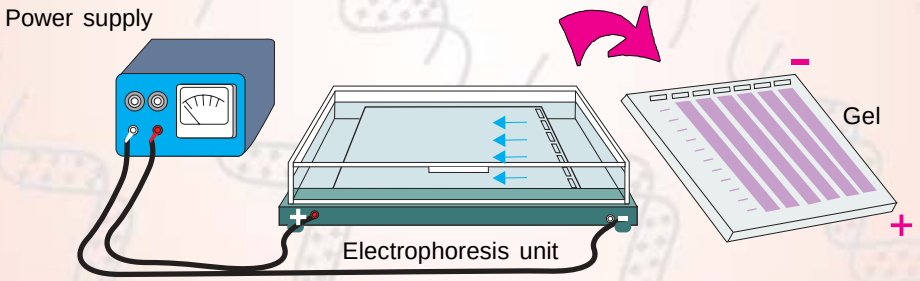
Of the three types of restriction enzymes, types I and III cut the double-stranded DNA outside the target sequence. In contrast, type II restriction enzymes identify specific sequences of 4, 5 or 6 base pairs and cut inside this sequence. Because of their features, all three types have become essential for recombinant DNA technology.

Enzymes may cut a given DNA sequence, leaving staggered (or 'sticky') ends that allow hydrogen-bonding to a complementary sequence or blunt ends. If two fragments of DNA are cut with the same enzyme, fragments with the same complementary sticky ends will be produced and alternative fragments may attach.

Restriction enzymes are commercially available, usually furnished with the appropriate reaction buffer and information about reaction conditions and temperatures.

Nucleic acid electrophoresis

A method to separate DNA fragments to allow their visualization and/or identification



Adapted from Griffiths *et al.* 1996

After digestion with a restriction enzyme, the DNA molecule is converted into a collection of restriction fragments. These fragments may be separated by size by running them through an agarose or acrylamide gel.

To obtain the separation, the mixture of DNA fragments and leftover restriction enzyme is placed in wells formed at one edge of the gel. The gel is then subjected to an electrical field, forcing the migration of DNA fragments according to their size, with large fragments migrating more slowly than short fragments. DNA molecules, negatively charged at neutral pH, migrate towards the anode. Agarose gels (0.8% to 2.0% agarose) are most useful for separating DNA fragments that range in size from 300 to 10,000 bp. Acrylamide gels (3.5% to 20% acrylamide) are most useful for fragments ranging between 20 and 1000 bp in size.

Visualisation of DNA fragments after electrophoresis is achieved by staining with ethidium bromide, a molecule that moves into the bases of the DNA and can fluoresce an orange colour under UV light.

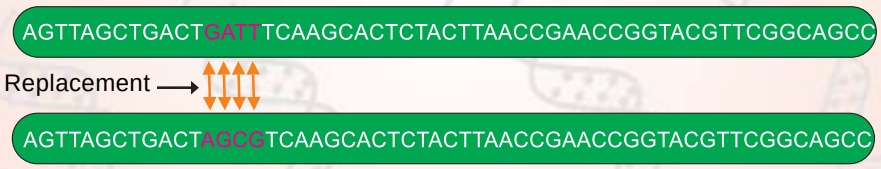
Migration distance is proportional to the logarithm of the number of bases. The actual size of the fragments obtained can therefore be calculated in relation to the mobility of DNA fragments of known size.

DNA polymorphism

- Various events may give rise to variants, more or less complex, in the DNA sequence. Such variants are usually described as polymorphisms
- Polymorphism is translated into differences in genotype—as evidenced in diverse band profiles when detected with an appropriate procedure—and perhaps phenotype
- Several events can produce polymorphisms:
  - Point mutations
  - Insertions or deletions
  - Rearrangements

Point mutations

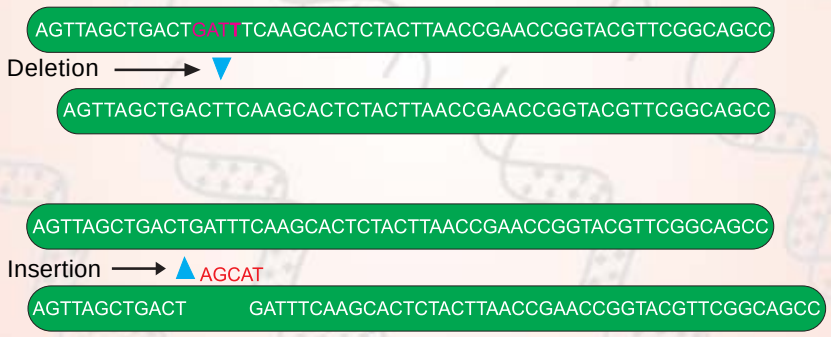
Point mutations occur when a base in the DNA sequence is replaced by another. The length of the DNA sequence does not change



Point mutations can occur in one base only or in a few bases at the same location. In the diagram above, four bases of the original chromosome sequence (top) are replaced by four alternative bases. Because the original number of the bases does not change, the sequence's total length does not alter.

Insertions or deletions

Insertions or deletions are the addition or the disappearance of several bases in the DNA sequence. The molecule length changes

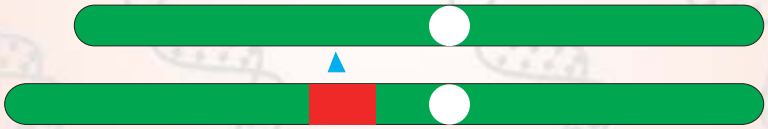


The top half of the diagram illustrates a deletion: some bases are lost and the resulting DNA fragment becomes shorter.

The bottom half of the diagram illustrates an insertion: some bases are introduced into a section of a DNA sequence. The original sequence thus becomes longer according to the number of bases being inserted.

Rearrangements

Chromosomal rearrangements occur through genetic recombination or insertion of transposable elements. The molecule length may or may not change



Changes in the sequence of the DNA may also occur through rearrangements, such as a segment flipping over. In these instances, although the length of the DNA sequence may not change, its composition could change sufficiently for it to be observed as a polymorphism.

DNA isolation procedure in pictures

The following photographs illustrate various steps of the procedures for isolating DNA

The laboratory technician is harvesting a few, very young, tomato leaves for a microprep extraction. For a large prep DNA extraction, many more, larger leaves (about 10 g) would be harvested from much older plants.

Leaf tissue (for microprep DNA extractions) and buffer are homogenized in a 1.5-ml microcentrifuge tube, using a drill fitted with a plastic pestle. To increase efficiency, two drills may be used simultaneously. These can be operated by foot pedals, like those used for sewing machines.

Leaf tissue for large prep DNA extractions is homogenized with DNA extraction buffer in standard kitchen blenders. Although not needed for safety, gloves and a laboratory apron may be worn to protect clothing and skin, as the procedure can be messy.

The mixture of leaf tissue and buffer is poured from the blender, through cheesecloth, into centrifuge bottles packed in ice. The cheesecloth is squeezed to get as much liquid as possible while filtering out large pieces of leaf tissue, which are then discarded, together with the filter.

After centrifuging and re-suspending the DNA pellet (which is still green and contains some leaf material), the mixture is transferred to a new tube and chloroform is added. This step should be performed in a ventilation hood, and safety gloves and a laboratory coat worn.

After inverting the tubes to gently mix in the chloroform, the tubes are centrifuged to separate out the DNA.

The lighter layer containing the DNA is now on the top, and can be transferred into a clean tube. The lower layer contains unwanted leaf tissue, cell walls and other residues, and is discarded.

Alcohol is added to precipitate out the DNA, which, on gentle inversion, usually comes together as a string-like substance. The DNA can then be either removed with a hook or spun down. It is then washed and re-suspended.

In summary

- The bricks of DNA are nucleotides, assembled in a double helix to form the DNA chain
- The DNA molecule becomes increasingly more folded as the nucleotide sequence forms the chromosome
- DNA is organized in different classes: single copy, multiple copy and spacer
- Mitochondria and chloroplasts have their own DNA molecule
- Several events give rise to polymorphisms in the DNA molecule: point mutations, insertions, deletions and rearrangements

By now you should know

- The components of a nucleotide
- How nucleotides form the DNA double helix
- The different classes of DNA
- The main features of cytoplasmic DNA
- What a restriction enzyme is
- How polymorphisms in the DNA chain are produced

Basic references

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Next

DNA-based technologies
Restriction fragment length polymorphisms (RFLPs)

- DNA-based technologies
  - PCR-based technologies
- Complementary technologies
- Final considerations
- Glossary