



DNA: THE INDISPENSIBLE FORENSIC SCIENCE TOOL

Introduction

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- **Portions of the DNA structure are as unique to each individual as fingerprints.**
- **The gene is the fundamental unit of heredity.**
- **Each gene is actually composed of DNA specifically designed to carry the task of controlling the genetic traits of our cells.**
- **DNA is constructed as a very large molecule made by linking a series of repeating units called nucleotides.**
- **A nucleotide is composed of a sugar, a phosphorous-containing group, and a nitrogen-containing molecule called a base.**

The Bases

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- **Four types of bases are associated with the DNA structure: adenine (A), guanine (G), cytosine (C), and thymine (T).**
- **The bases on each strand are properly aligned in a double-helix configuration, which is two strands of DNA coiled together.**
- **As a result, adenine pairs with thymine and guanine pairs with cytosine.**
- **This concept is known as base pairing.**
- **The order of the bases is what distinguishes different DNA strands.**

DNA at Work

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- DNA directs the production of proteins, which are made by combining amino acids.
- The sequence of amino acids in a protein chain determines the shape and function of the protein.
- Each group of three nucleotides in a DNA sequence codes for a particular amino acid.
 - Example: G-A-G codes for the amino acid glutamine, while C-G-T codes for alanine.
- If a nucleotide is “changed”, for example a T is substituted for A and G-A-G becomes G-T-G, the “wrong” amino acid is placed in the protein (in this case glutamine is replaced with valine).
- As a result, the protein may not function correctly and this is the basis for many diseases and health issues.

DNA Replication

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- DNA duplicates itself prior to cell division.
- DNA replication begins with the unwinding of the DNA strands of the double helix.
- Each strand is now exposed to a collection of free nucleotides that will be used to recreate the double helix, letter by letter, using base pairing.
- Many enzymes and proteins, such as DNA polymerases, are involved in unwinding the DNA, keeping the DNA strands apart, and assembling the new DNA strands.
- Polymerase chain reaction (PCR) is a technique for replicating small quantities of DNA or broken pieces of DNA found at a crime scene, outside a living cell.
- The ability to multiply small bits of DNA now means that sample size is no longer a limitation in characterizing DNA recovered at a crime scene.

Recombinant DNA

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- **Recombinant DNA** relies on the ability of certain chemicals, known as restriction enzymes, to cut DNA into fragments that can later be incorporated into another DNA strand.
- Restriction enzymes can be thought of as highly specialized scissors that cut a DNA molecule when it recognizes a specific sequence of bases.
- Once a portion of the DNA strand has been cut out with the aid of a restriction enzyme, the next step in the recombinant DNA process is to insert the isolated DNA segment into a foreign DNA strand, usually that of a bacterium.
- As the bacteria multiply rapidly, copies of the altered DNA are passed on to all descendants.

DNA Typing

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- **Portions of the DNA molecule contain sequences of bases that are repeated numerous times, known as tandem repeats.**
- **To a forensic scientist, these tandem repeats offer a means of distinguishing one individual from another through DNA typing.**
- **Tandem repeats seem to act as filler or spacers between the coding regions of DNA.**
- **What is important to understand is that all humans have the same type of repeats, but there is tremendous variation in the number of repeats each of us have.**

RFLP

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- Length differences associated with relatively long repeating DNA strands are called restriction fragment length polymorphisms (RFLP) and form the basis for one of the first DNA typing procedures.
- Typically, a core sequence consists of 15 to 35 bases in length and repeats itself up to a thousand times.
- The key to understanding DNA typing lies in the knowledge that numerous possibilities exist for the number of times a particular sequence of base letters can repeat itself on a DNA strand.

A Positive RFLP Test

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- Once the DNA molecules have been cut up by a restriction enzyme, the resulting fragments are sorted out by electrophoresis.
- The smaller DNA fragments will move at a faster rate on the gel plate than the larger ones.
- The fragments are then transferred to a nylon membrane in a process called Southern blotting.
- To visualize the RFLPs, the nylon sheet is treated with radioactive probes containing a base sequence complementary to the RFLPs being identified (a process called hybridization).

A Positive RFLP Test

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- Next, the nylon sheet is placed against X-ray film and exposed for several days.
- When the film is processed, bands appear where radioactive probes stuck to fragments on the nylon sheet.
- A typical DNA fragment pattern will show two bands (one RFLP from each chromosome).
- When comparing the DNA fragment patterns of two or more specimens, one merely looks for a match between the band sets.
- A high degree of discrimination can be achieved by using a number of different probes and combining their frequencies.

PCR Testing

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- **Polymerase chain reaction is the outgrowth of knowledge gained from an understanding of how DNA strands naturally replicate within a cell.**
- **For the forensic scientist, PCR offers a distinct advantage in that it can amplify minute quantities of DNA many millions of times.**
- **First, the DNA is heated to separate it.**
- **Second, primers (short strands of DNA used to target specific regions of DNA for replication) are added which hybridize with the strands.**
- **Third, DNA polymerase and free nucleotides are added to rebuild each of the separated strands.**
- **Now, this process is repeated 25 to 30 times.**

PCR and RFLP

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- PCR technology cannot be applied to RFLP DNA typing.
- The RFLP strands are too long, often numbering in the thousands of bases.
- PCR is best used with DNA strands that are no longer than a couple of hundred bases.

PCR Advantages

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- **One advantage in moving to shorter DNA strands is that they would be expected to be more stable and less subject to degradation brought about by adverse environmental conditions.**
- **The long RFLP strands tend to readily break apart under the adverse conditions not uncommon at crime scenes.**
- **PCR also offers the advantage in that it can amplify minute quantities of DNA, thus overcoming the limited sample size problem often associated with crime scene evidence.**

Short Tandem Repeats

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- The latest method of DNA typing, short tandem repeat (STR) analysis, has emerged as the most successful and widely used DNA profiling procedure.
- STRs are locations on the chromosome that contain short sequences that repeat themselves within the DNA molecule.
- They serve as useful markers for identification because they are found in great abundance throughout the human genome.

STR Advantages

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- **STRs normally consist of repeating sequences of 3 to 7 bases in length, and the entire strand of an STR is also very short, less than 450 bases in length.**
- **This means that STRs are much less susceptible to degradation and may often be recovered from bodies or stains that have been subjected to extreme decomposition.**
- **Also, because of their shortness, STRs are ideal candidates for multiplication by PCR, thus overcoming the previously mentioned limited-sample-size problem often associated with crime-scene evidence.**

The Power of STR

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- **What makes STRs so attractive to forensic scientists is that hundreds of different types of STRs are found in human genes.**
- **The more STRs one can characterize, the smaller will be the percentage of the population from which a particular combination of STRs can emanate.**
- **This gives rise to the concept of multiplexing.**
- **Using the technology of PCR, one can simultaneously extract and amplify a combination of different STRs.**

Standardizing STR Testing

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- **Currently, U.S. crime laboratories have standardized on 13 STRs for entry into a national database (CODIS).**
- **A high degree of discrimination and even individualization can be attained by analyzing a combination of STRs (multiplexing) and determining the product of their frequencies.**
- **With STR, as little as 125 picograms of DNA is required for analysis.**
- **This is 100 times less than that normally required for RFLP analysis.**

Y- STR

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- **Another tool available in the arsenal of the DNA analyst is the ability to type STRs located on the Y chromosome, which is male specific.**
- **More than 20 different Y-STR markers have been identified.**
- **Y-STRs will prove useful when multiple males are involved in a sexual assault.**
- **A Y-STR analysis will have only one band or peak, rather than the conventional STR which is derived from two chromosomes and has two bands or peaks.**
- **The Y-STR is therefore less complicated in appearance and interpretation.**

Mitochondrial DNA

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- Another type of DNA used for individual characterization is mitochondrial DNA.
- Mitochondrial DNA (mDNA) is located outside the cell's nucleus and is inherited from the mother.
- Mitochondria are structures found in all our cells used to provide energy that our bodies need to function.
- A single mitochondria contains several loops of DNA.

Mitochondrial DNA Testing

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- Mitochondrial DNA typing does not approach STR analysis in its discrimination power and thus is best reserved for samples, such as hair, for which STR analysis may not be possible.
- Forensic analysis of mDNA is more rigorous, time consuming, and costly when compared to nuclear DNA analysis.
- Also, all individuals of the same maternal lineage will be indistinguishable by mDNA analysis.
- Two regions of mDNA have been found to be highly variable and a procedure known as sequencing is used to determine the order of base pairs.

CODIS

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- Perhaps the most significant tool to arise from DNA typing is the ability to compare DNA types recovered from crime scene evidence to those of convicted sex offenders and other convicted criminals.
- CODIS (Combined DNA Index System) is a computer software program developed by the FBI that maintains local, state, and national databases of DNA profiles from convicted offenders, unsolved crime scene evidence, and profiles of missing persons.

Packaging Biological Evidence

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- Before the collection of biological evidence begins, it is important that it be photographed and recorded on sketches.
- Wearing disposable latex gloves while handling the evidence is required.
- Clothing from victim and suspect with blood evidence must be collected.
- The packaging of biological evidence in plastic or airtight containers must be avoided because the accumulation of residual moisture could contribute to the growth of DNA-destroying bacteria and fungi.

Packaging Biological Evidence

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- Each stained article should be packaged separately in a paper bag or in a well-ventilated box.
- Dried blood is best removed from a surface by using a sterile cotton swab lightly moistened with distilled water that is air dried before being placed in a swab box, then a paper or manila envelope.
- All biological evidence should be refrigerated or stored in a cool location until delivery to the laboratory.
- Standard/reference DNA specimens must also be collected, such as blood or the buccal swab (swabbing the mouth and cheek).

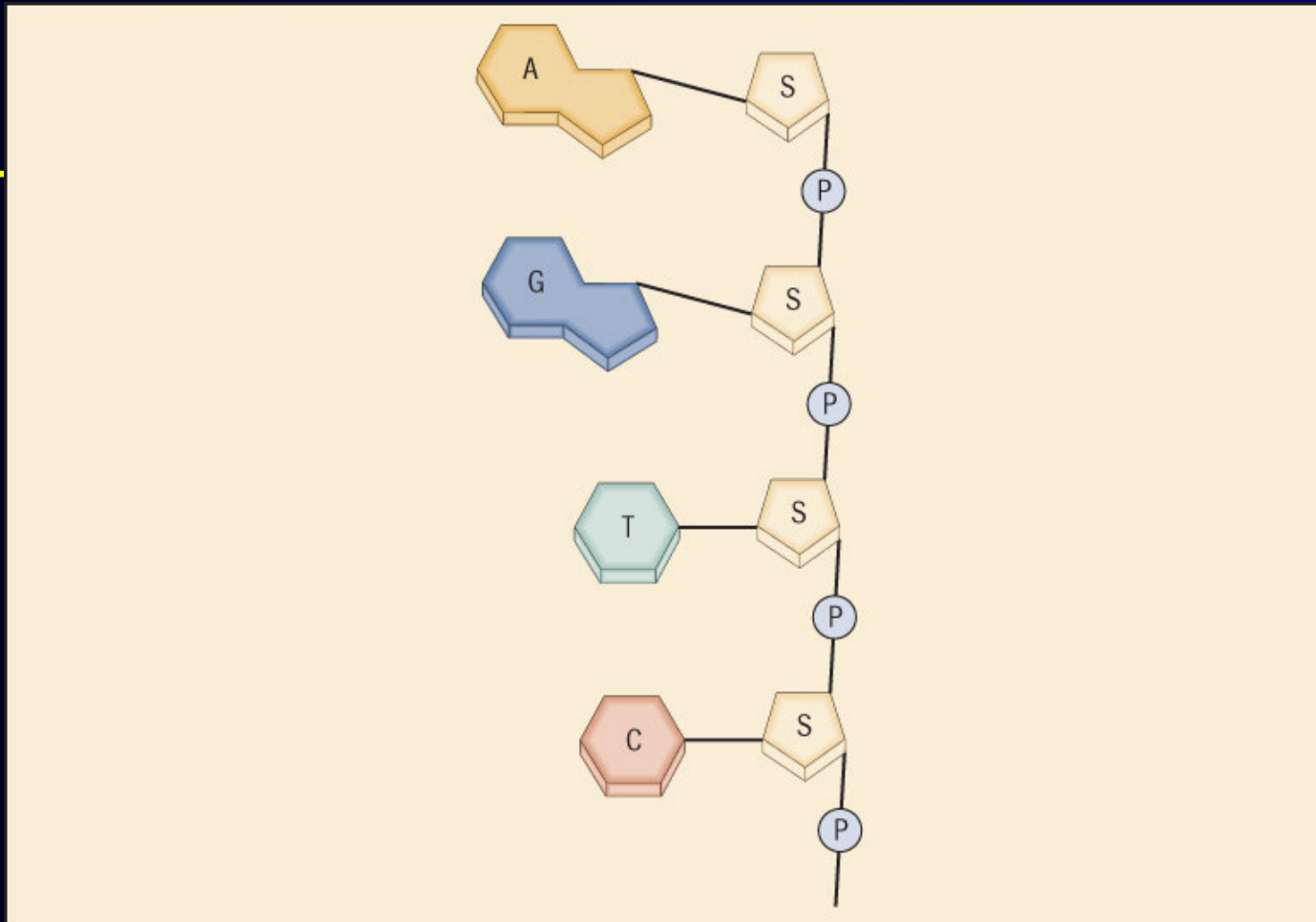


Figure 13–1 How nucleotides can be linked to form a DNA strand. S designates the sugar component, which is joined with phosphate groups (P) to form the backbone of DNA. Projecting from the backbone are four bases: A, adenine; G, guanine; T, thymine; and C, cytosine.

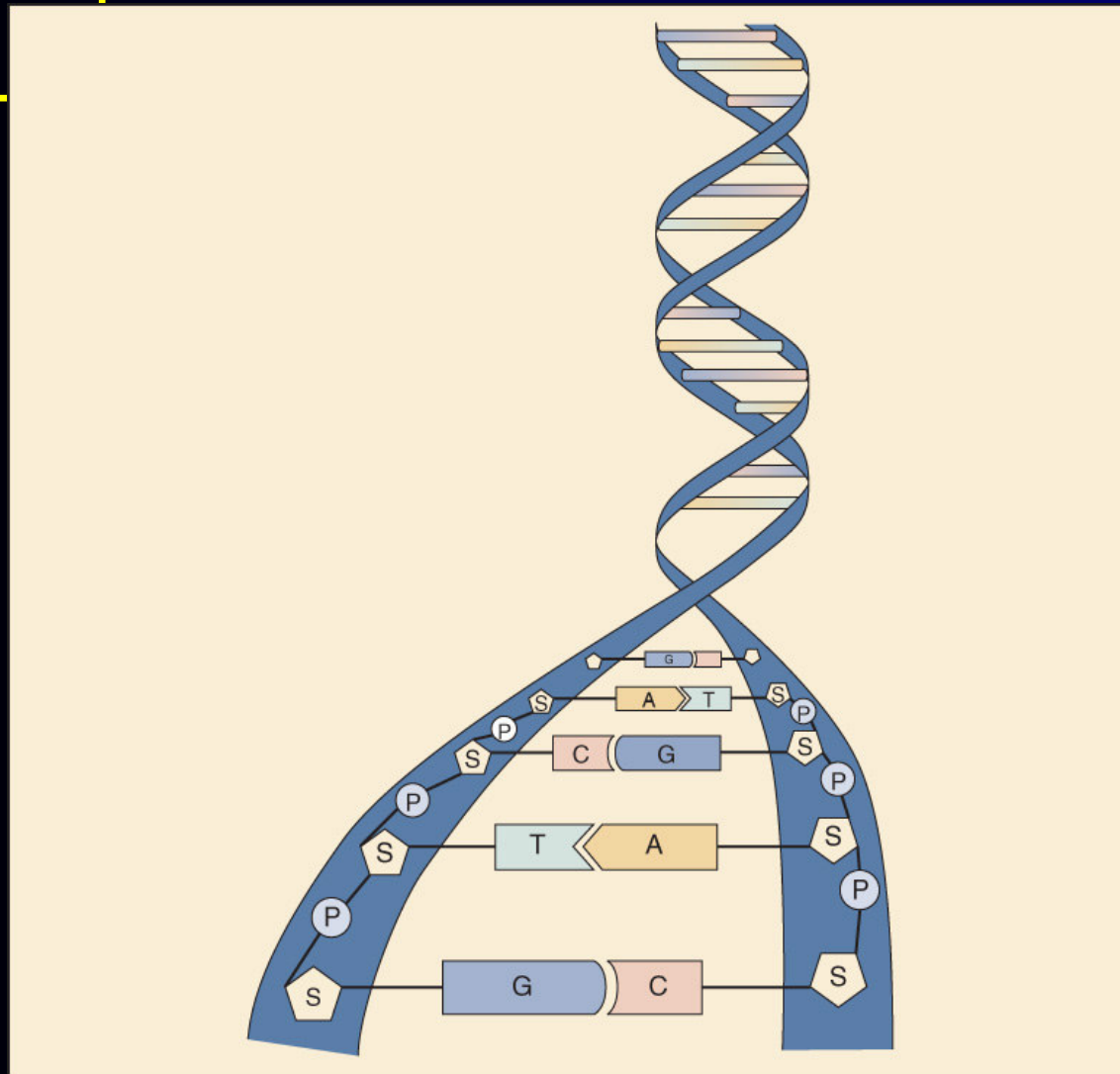


Figure 13–2 A
representation of a
DNA double helix.
Notice how bases **G**
and **C** pair with each
other, as do bases **A**
and **T**. This is the only
arrangement in which
two DNA strands can
align with each other
in a double-helix
configuration.

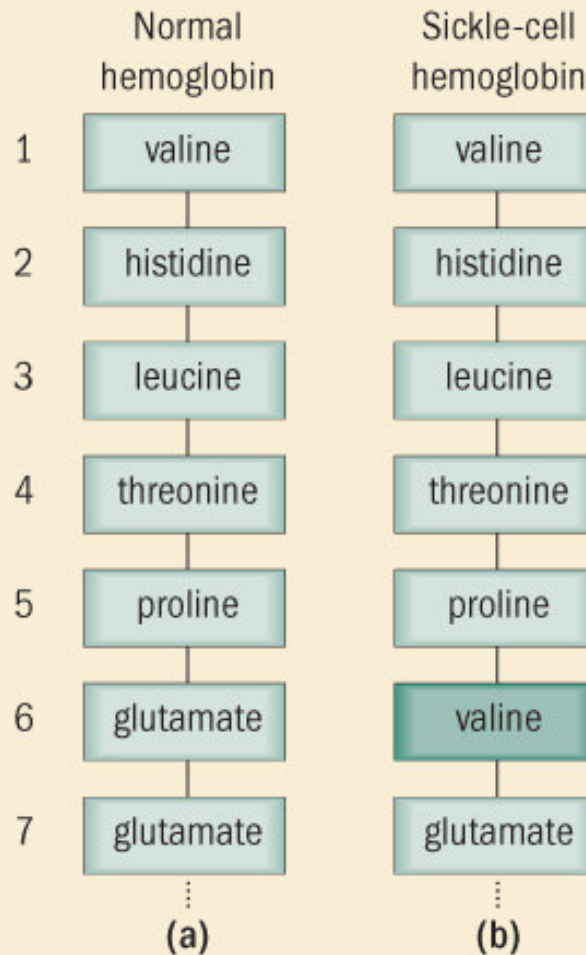


Figure 13–3 (a) A string of amino acids composes one of the protein chains of hemoglobin. (b) Substitution of just one amino acid for another in the protein chain results in sickle-cell hemoglobin.

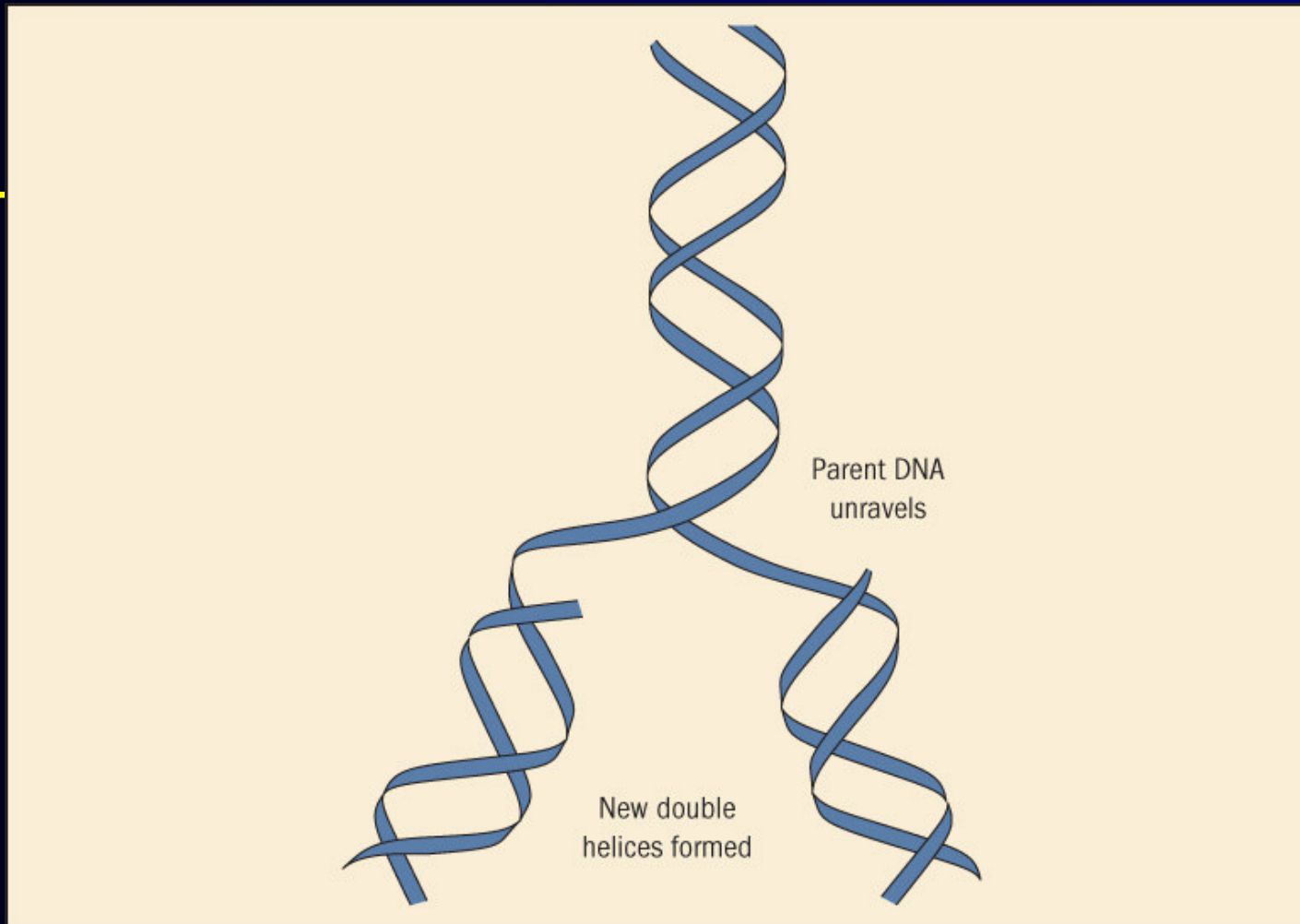
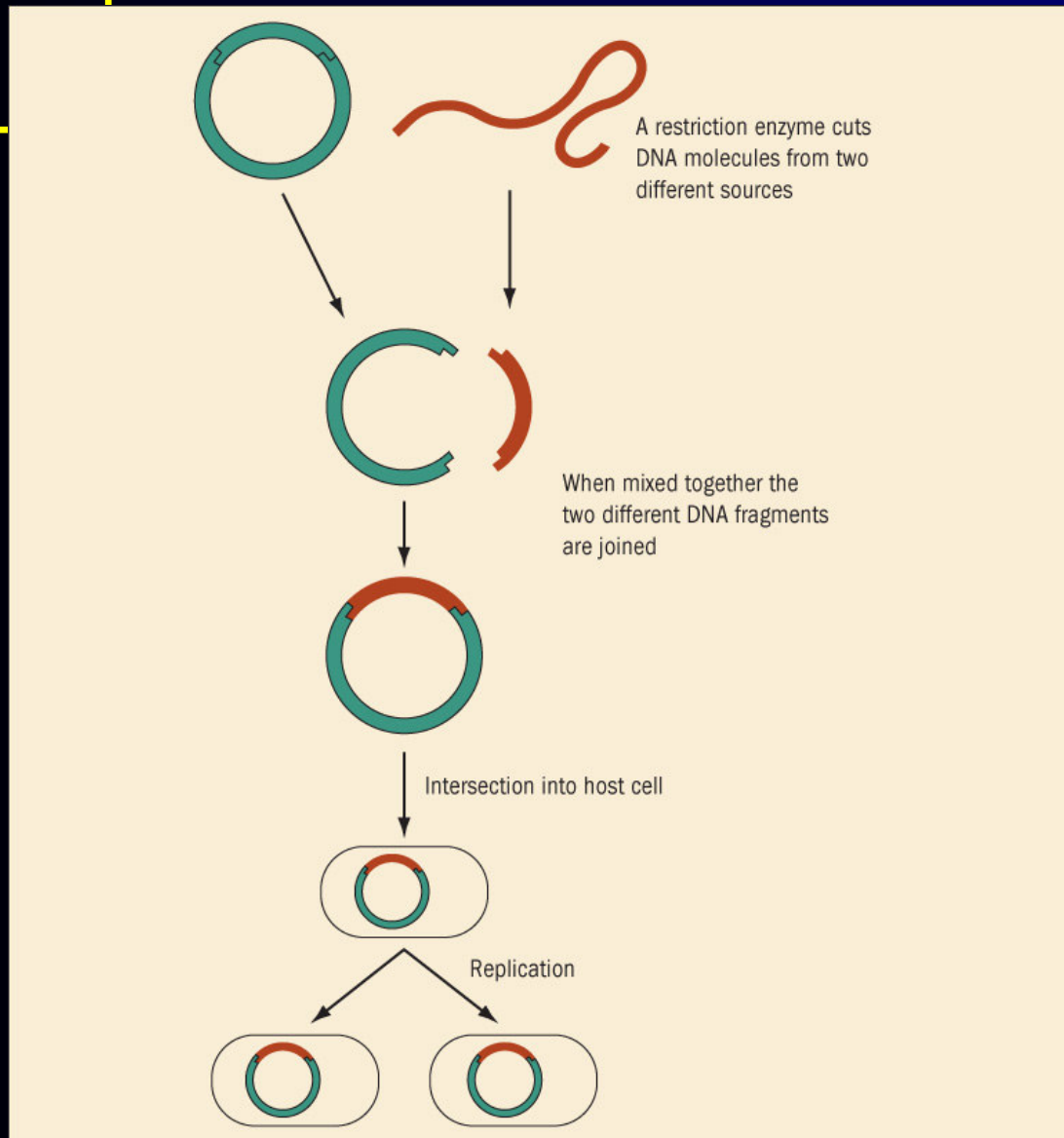


Figure 13–4 Replication of DNA. The strands of the original DNA molecule are separated, and two new strands are assembled.

Figure 13–6 The joining of DNA from two different sources via recombinant DNA technology.



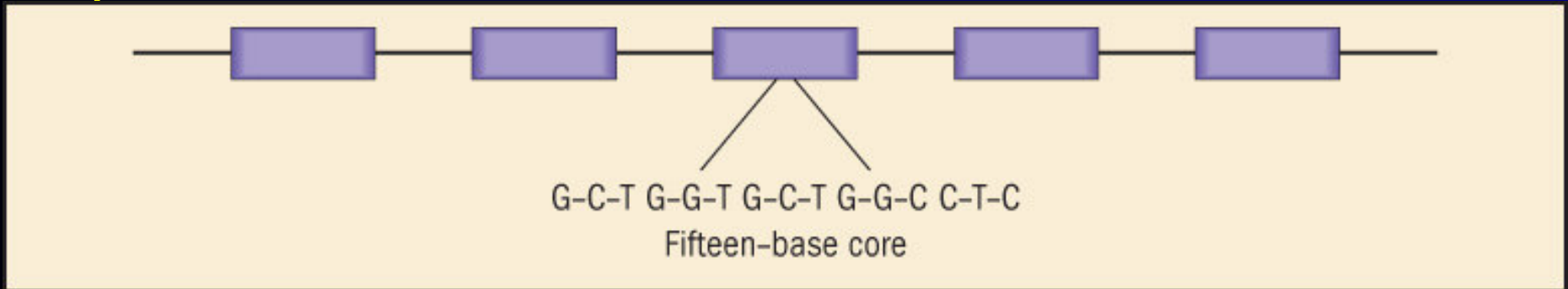


Figure 13–7 A DNA segment consisting of a series of repeating DNA units. In this illustration, the fifteen-base core can repeat itself hundreds of times. The entire DNA segment is typically hundreds to thousands of bases long.

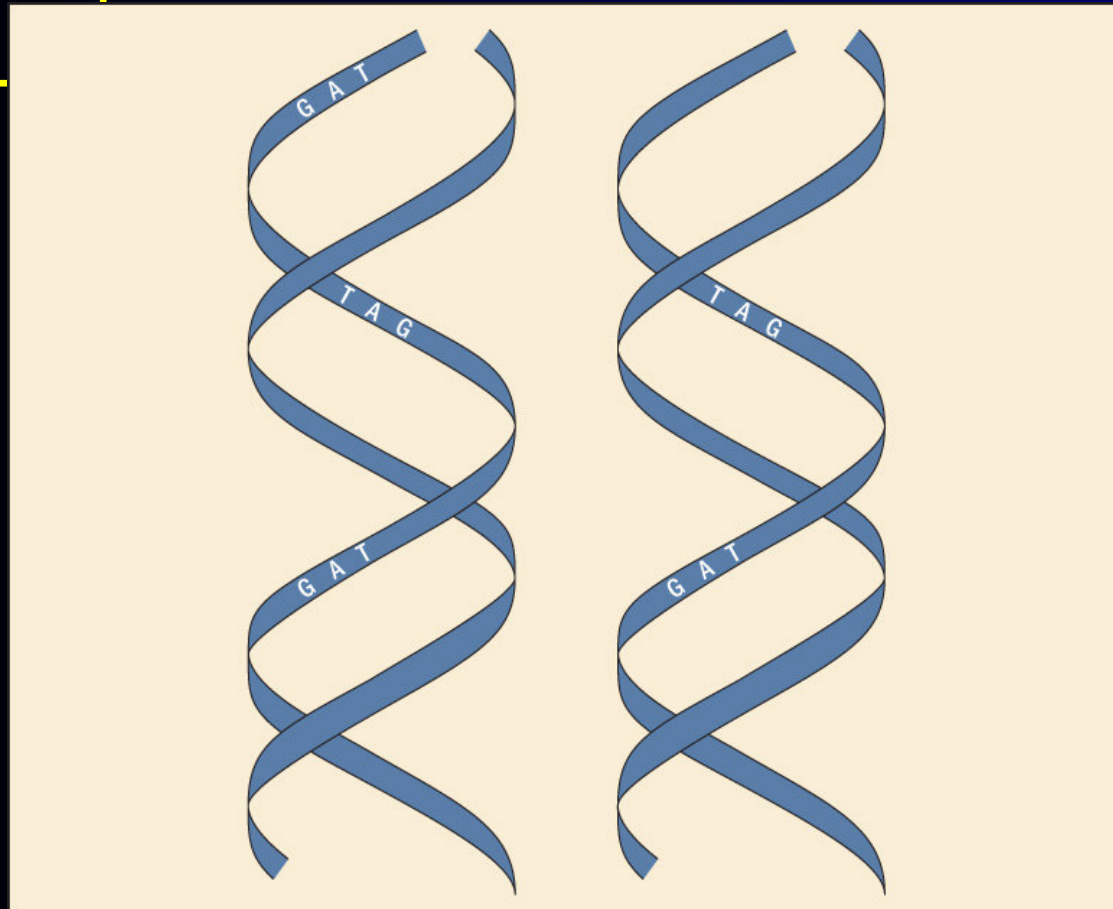
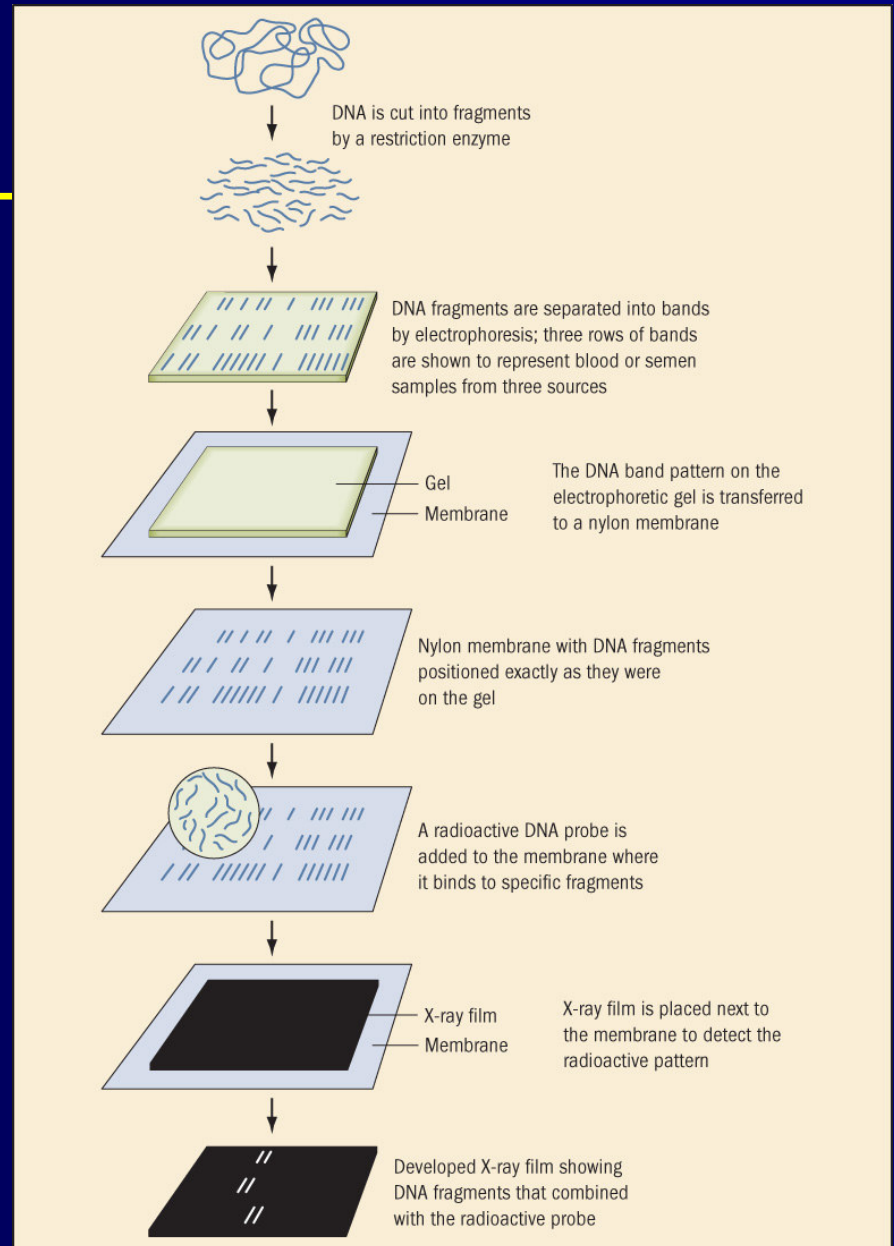


Figure 13–8 Intertwined strands of DNA representing segments of two chromosomes. Note that the chromosome segment on the left contains three repeating sequences of T–A–G, while the chromosome segment on the right has two repeating sequences of T–A–G.

Figure 13-9 The DNA RFLP typing process.



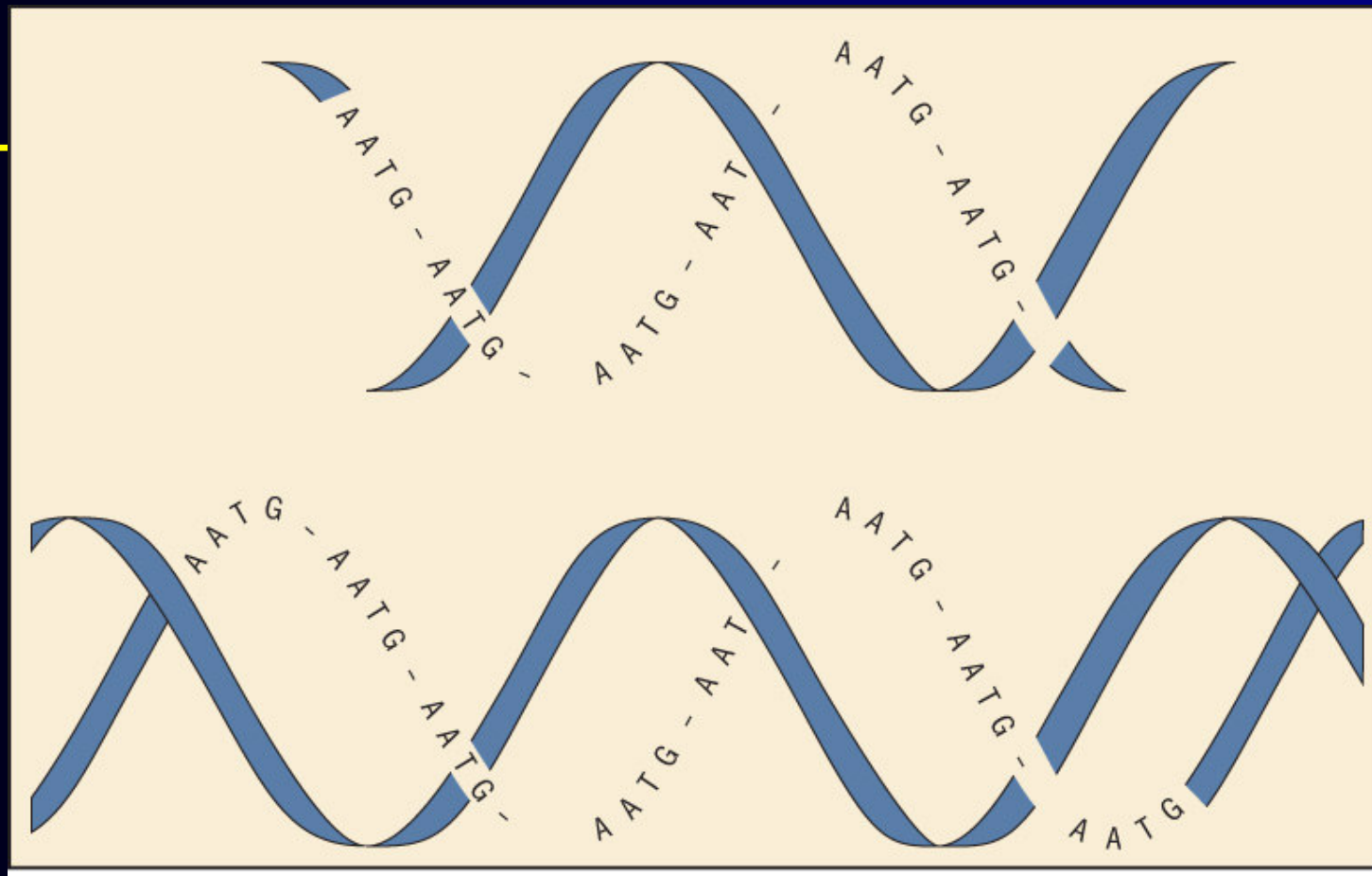
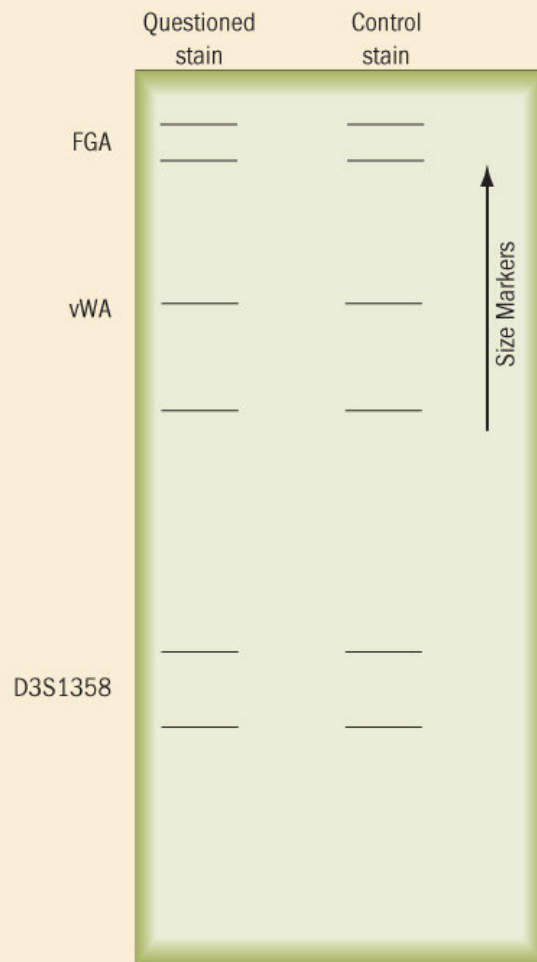


Figure 13–12 Variants of the short tandem repeat TH01. The upper DNA strand contains six repeats of the sequence A–A–T–G; the lower DNA strand contains eight repeats of the sequence A–A–T–G.

Figure 13-13 Triplex system containing three loci: FGA, vWA, and D3S1358, indicating a match between the questioned and the standard/reference stains.



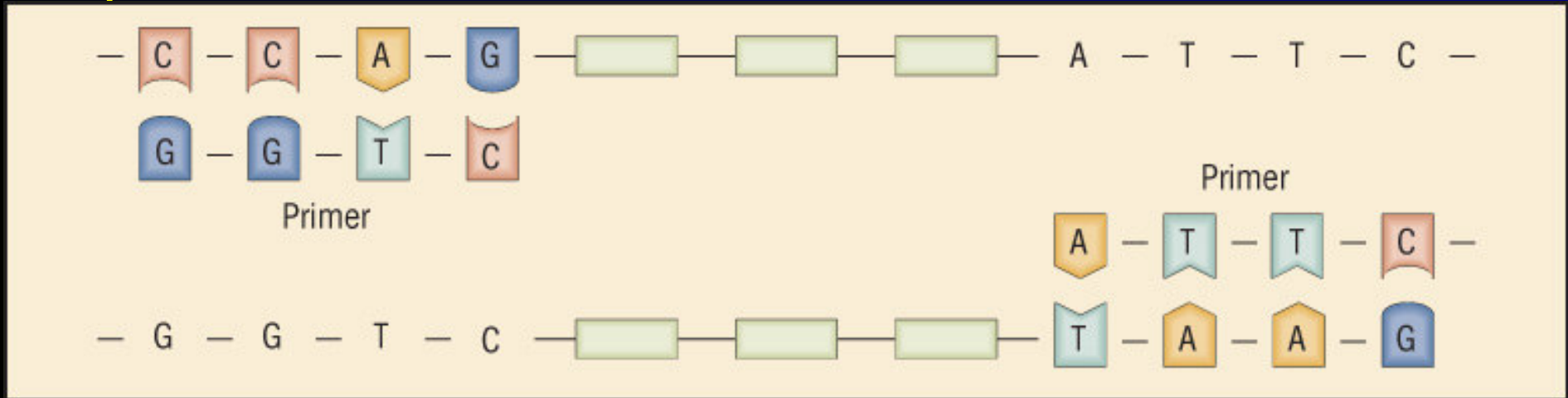


Figure 13–14 Appropriate primers flanking the repeat units of a DNA segment must be selected and put in place in order to initiate the PCR process.

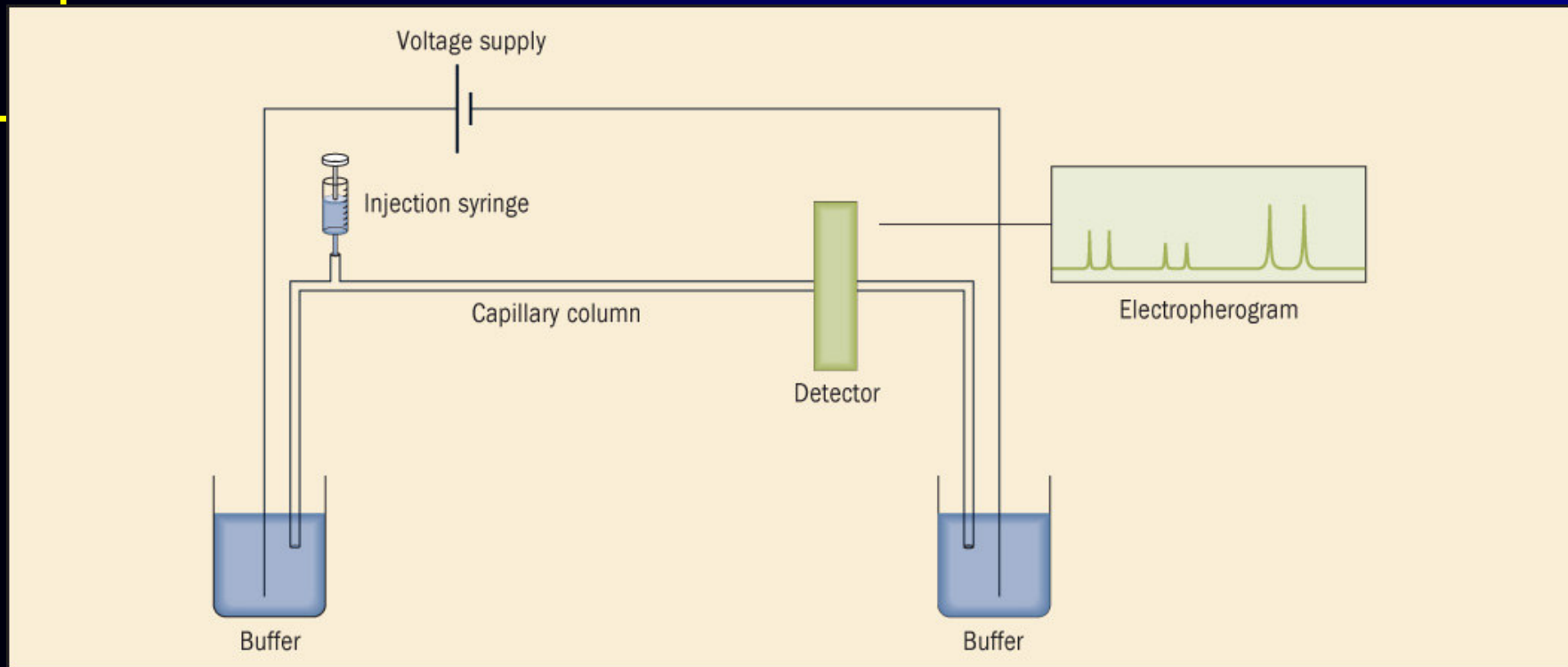


Figure 13–15 Capillary electrophoresis technology has evolved from the traditional flat gel electrophoresis approach. The separation of DNA segments is carried out on the interior wall of a glass capillary tube that is kept at a constant voltage. The size of the DNA fragments determines the speed at which they move through the column. This figure illustrates the separation of three sets of STRs (triplexing).

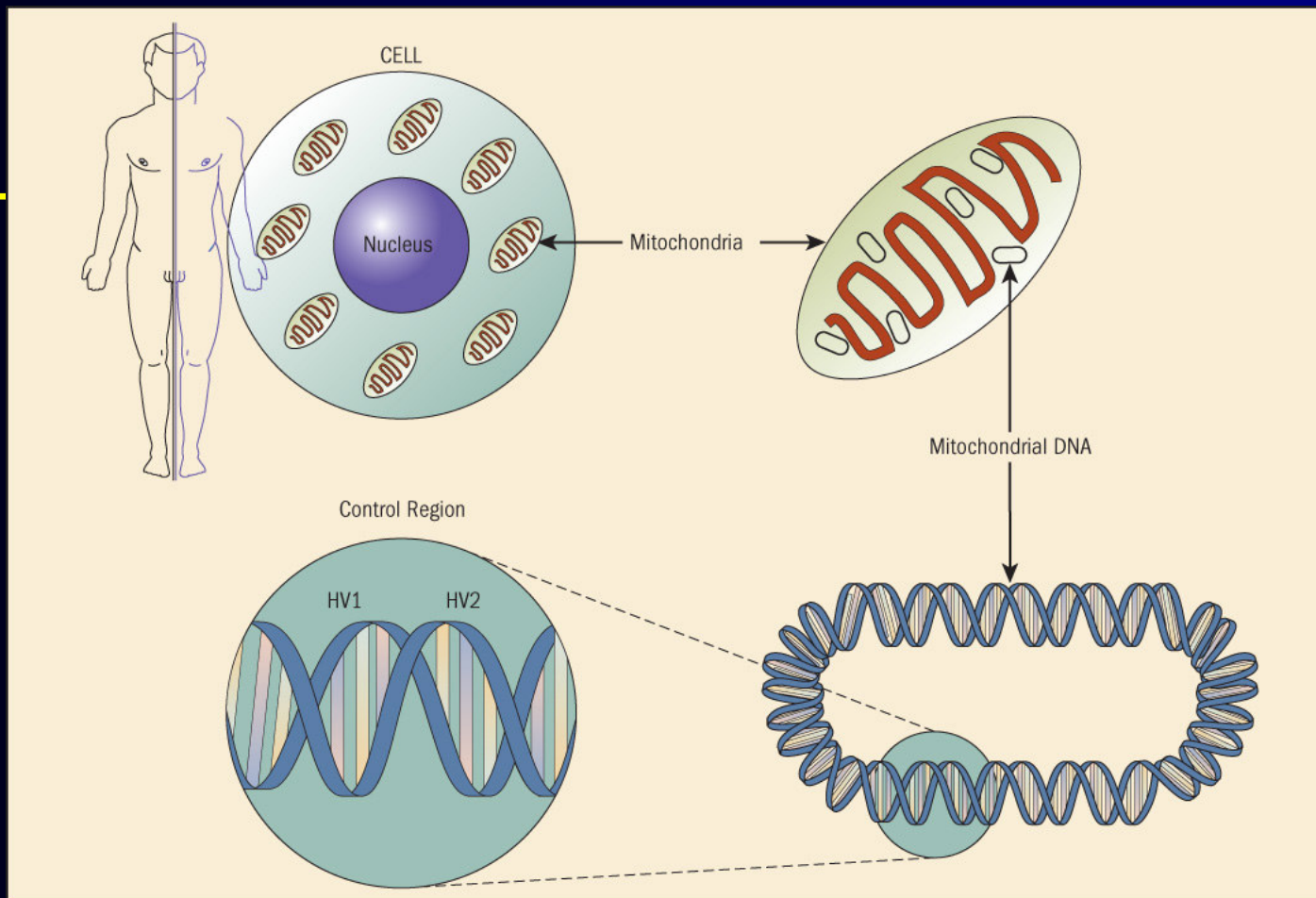


Figure 13–17 Every cell in the body contains hundreds of mitochondria, which provide energy to the cell. Each mitochondrion contains numerous copies of DNA shaped in the form of a loop. Distinctive differences between individuals in their mitochondrial DNA makeup are found in two specific segments of the control region on the DNA loop known as HV1 and HV2.