Consider the relationship between DNA sequence and DNA fingerprinting.

It is observed that human chromosomes exhibit a one base difference in sequence for every 250- 500 bases. These base differences may or may not give rise to differences in amino acid sequences of proteins because not all segments of DNA code for proteins and there is more than one codon for most amino acids.

Single base differences can create or abolish a recognition site for a restriction enzyme as suggested by Fig 28-50.

If a specific recognition base sequence is present, the restriction enzyme recognizing that site will cleave the DNA molecule and result in fragments of specific length.

If the site is absent due to a base difference, different fragment lengths will result.

Differences in base sequence between individuals in the population are referred to as polymorphism.

Restriction fragment length polymorphism (RFLP) refers to the different lengths of DNA fragments observed between individuals in a population.

The analysis of individual differences in the pattern (lengths) of restriction fragments is commonly known as DNA fingerprinting.

The analysis involves many steps as described in Fig 6-1.

DNA is first extracted from blood or other tissue containing nucleated cells.

The DNA is treated with a restriction enzyme and the fragments separated by agarose or polyacrylamide gel electrophoresis.

The DNA fragments are transferred to a nylon membrane in the same order as they appear in the gel by a process called Southern blotting as shown in the Fig.

The blotting uses an alkaline solution that separates the strands of each DNA fragment. The strands of each DNA fragment bind to positively charged groups in the nylon membrane.

The membrane with bound fragment strands is then exposed to a solution containing a ^{32}P labeled oligonucleotide whose sequence is complementary to a segment of a known sequence in DNA.

The oligonucleotide will hybridize (bind) to that fragment containing the complementary sequence.

The membrane is washed to remove nonspecifically bound ^{32}P labeled oligonucleotide and the membrane exposed to photographic film to locate the fragment on the membrane.

The fragment length is determined from the position on the gel compared to DNA markers of known length.

In most cases 2 bands, corresponding to each parental gene, are observed for each oligonucleotide probe as shown in Fig 28-51.

Humans have 23 pairs of chromosomes (DNA molecules with bound protein).

One chromosome from each pair comes from each parent. Thus a gene (allele) coding for a particular protein is inherited from each parent.

As shown in Fig 28-51, the two genes may be different (heterozygous) with regard to sequence, represented by CD, for one parent or the two genes may be the same (homozygous) represented by AA.

The first generation child F1 will get one gene from each parent such as AC.

RFLP analysis shows two bands (of different length) in the autoradiogram for the CD parent and one band for the AA parent.

The AC child shows an A band and a C band, one fragment corresponding to each gene inherited from the parent.

DNA fingerprinting is now widely used in forensics, establishing the basis for familial diseases and in parentage testing.

The handout page indicates a typical autoradiogram of an RFLP used to evaluate parentage.

Each RFLP analysis for a particular gene fragment includes a control (a previously determined sample to indicate that the restriction digestion, Southern blotting, and electrophoresis were done correctly), DNA markers (fragments of known length measured in kilobases), mother, child, alleged father, and lastly alleged father and child DNA combined.

The autoradiogram has four cases. Two for which a band position of the alleged father matches that of one of the child's bands and two for which they do not match.

The two cases for which there is no match excludes the alleged father from being the father.

The two cases for which bands match indicate that the alleged father may be the father.

A paternity index is associated with each match as indicated on the evaluation reports.

The paternity index is the ratio of individual analyses done on the population to those that have a particular fragment-

Usually the DNA fragments resulting from a restriction digest (such as HAEIII) are separately analyzed with several ^{32}P labeled oligonucleotide probes (D14S13CMM101, D17S26EFD52, etc.).

Each gene fragment analyzed will have a different paternity index.

From these, a combined paternity index is reported which is a product of the individual indexes for each RFLP analysis-

The data is also reported as a probability. In the case indicated-

Today most genetic fingerprinting analyzes regions of our genome referred to as minisatellites that have highly variable repeating sequences.

The minisatellites have short tandem repeats (STR) most commonly 3-5 bases that are repeated several times such as $(GTCA)_n$.

Polymorphism is observed between the numbers of repeats (n) in each STR region.

Typically 5-20% of individuals share the same number of repeats at each STR region.

By examining the number of repeats in many minisatellites, it is possible to establish a unique profile for an individual.

Genetic fingerprinting of STR uses the polymerase chain reaction (PCR) described in Fig. 9-16 to generate multiple copies of a minisatellite.

The length of the minisatellite and thus the number of repeats is determined by comparing the time of elution of the minisatellite DNA in capillary gel electrophoresis to the time of elution of DNA strands of known length.

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