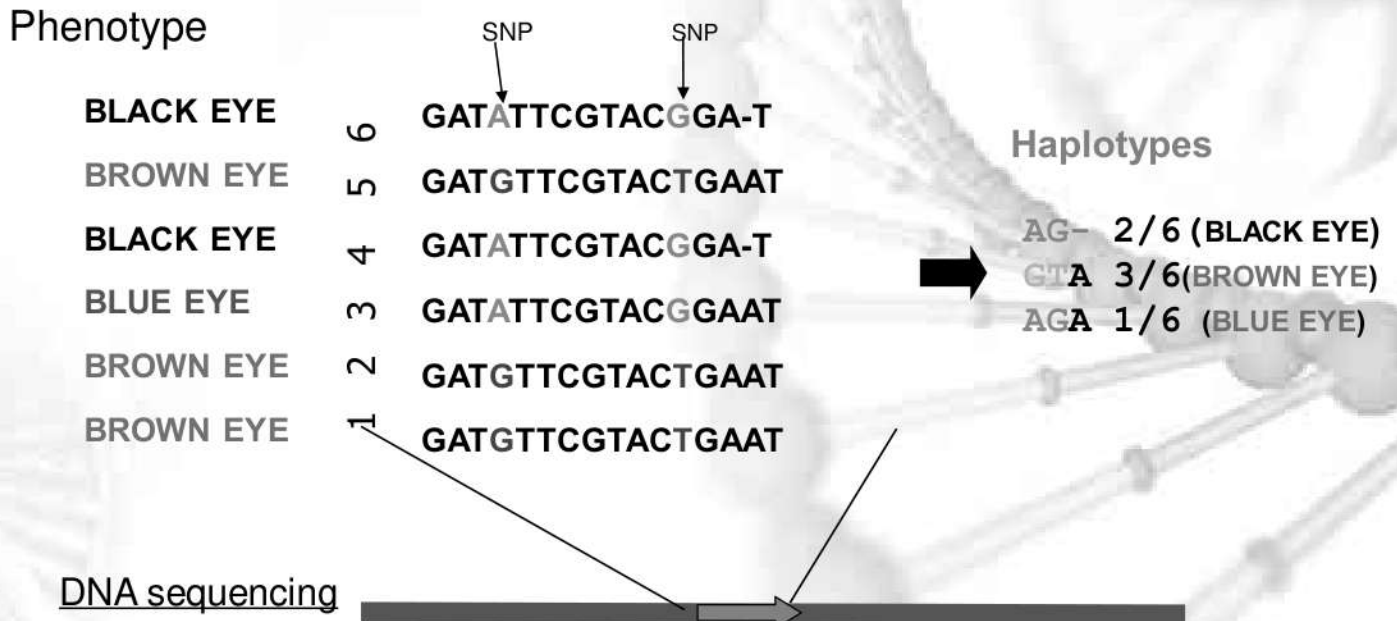


SNP-Haplotype



HAPLOTYPE CORRELATION WITH PHENOTYPE

- The “Haplotype centric” approach combines the information of adjacent SNPs into composite multilocus haplotypes
- Haplotypes are not only more informative but also capture the regional LD information, which is assumed to be robust and powerful
- Association of haplotype frequencies with the presence of desired phenotypic frequencies in the population will help in utilizing the maximum potential of SNP as a marker.

Some important SNP database Resources

- 1. dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>)
- LocusLink (<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi>)
- 2. TSC (<http://snp.cshl.org/>)
- 3. SNPper (<http://snpper.chip.org/bio/>)
- 4. JSNP (<http://snp.ims.u-tokyo.ac.jp/search.html>)
- 5. GeneSNPs (<http://www.genome.utah.edu/genesnps/>)
- 6. HGVbase (<http://hgibase.cgb.ki.se/>)
- 7. PolyPhen (<http://dove.embl-heidelberg.de/PolyPhen/>)
- OMIM (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>)
- 8. Human SNP database
(<http://www-genome.wi.mit.edu/snp/human/>)

Lesson No.62

DNA Fingerprinting

- Synonyms...
- DNA profiling.
- DNA testing.
- DNA typing.
- Genetic fingerprinting

Introduction

- The process of DNA fingerprinting was developed by Professor Alec Jeffreys at Leicester University in 1984 as a form of genetic analysis. • It was first used in the law courts of England in 1987 to convict a man in a rape case. • It has now been used successfully in many crime and paternity cases in worldwide.



Although 99.9% of human DNA sequences are the same in every person, enough of the DNA is different to distinguish one individual from another, unless they are monozygotic twins.

- DNA profiling uses repetitive sequences that are highly variable, called variable number tandem repeats (VNTRs), particularly short tandem repeats (STRs). VNTR loci are very similar between closely related humans.
- The analysis of variable number of tandem repeats (VNTRs), to detect the degree of relatedness to another sequence of oligonucleotides, making them ideal for DNA fingerprinting.

Variable Number Tandem Repeats

- A Variable Number Tandem Repeat (or VNTR) is a location in a genome where a short nucleotide sequence is organized as a tandem repeat.
- These can be found on many chromosomes, and often show variations in length between individuals.
- Each variant acts as an inherited allele, allowing them to be used for personal or parental identification.

- There are two principal families of VNTRs:

- Microsatellites.

- Minisatellites.

Microsatellites, also known as Simple Sequence Repeats (SSRs) or short tandem repeats (STRs), are repeating sequences of 2-6 base pairs of DNA. •

A minisatellites (also referred as VNTR) is a section of DNA that consists of a short series of bases 10–60 base pairs. •

Their analysis is useful in genetics and biology research, forensics, and DNA fingerprinting.

Lesson No.63

DNA profiling process

- The process begins with a sample of an individual's DNA (typically called a "reference sample").
 - The most desirable method of collecting a reference sample is the use of a buccal swab, as this reduces the possibility of contamination.
 - When this is not available may need to be used to collect a sample of blood, saliva, semen, or other appropriate fluid or tissue from personal items (e.g. toothbrush, razor, etc.) or from stored samples (eg: banked sperm or biopsy tissue).

Procedure

- 1) Isolation of DNA :
 - DNA must be recovered from the cells or tissues of the body.
 - Only a small amount of tissue, like blood, hair, or skin, is needed.
 - For example, the amount of DNA found at the root of one hair is usually sufficient.

- 2) Cutting, sizing, and sorting :

- Special enzymes called restriction enzymes are used to cut the DNA at specific sites.
- For example, an enzyme called EcoR1, found in bacteria, will cut DNA only when the sequence 5'..GAATTC..3' occurs.
- The DNA pieces are sorted according to size by a sieving technique called electrophoresis.
- The DNA pieces are passed through a gel agarose. This technique is the DNA equivalent of screening sand through progressively finer mesh screens to determine particle sizes.

- 3) Transfer of DNA to nylon :

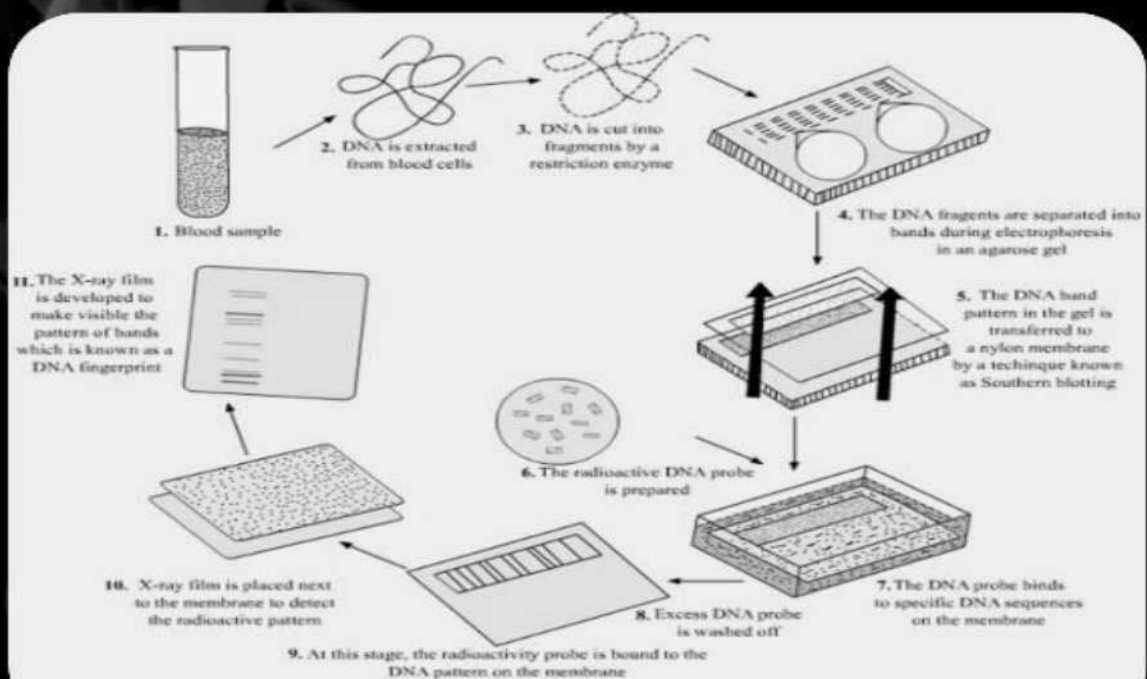
- The distribution of DNA pieces is transferred to a nylon sheet by placing the sheet on the gel and soaking them overnight.

- 4) Probing :

- Adding radioactive or colored probes to the nylon sheet produces a pattern called the DNA fingerprint.
- Each probe typically sticks in only one or two specific places on the nylon sheet.

- 5) DNA fingerprint :

- The final DNA fingerprint is built by using several probes (5-10 or more) simultaneously. It resembles the bar codes used by grocery store scanners.



Steps in DNA fingerprinting technique

Lesson No.64

Types of DN fingerprinting methods

- Types of DNA fingerprinting methods are...
- Electrophoresis.
- Polymerase chain reaction (PCR).
- Restriction fragment length polymorphism (RFLP).
- Random Amplified Polymorphic DNA(RAPD).
- Amplified fragment length polymorphism (AFLP).

Electrophoresis

- Electrophoresis is a separations technique that is based on the mobility of ions in an electric field. Positively charged ions migrate towards a negative electrode and negatively-charged ions migrate toward a positive electrode. Ions have different migration rates depending on their total charge, size, and shape, and can therefore be separated.

Polymerase chain reaction...

chain reaction (PCR) was developed

- The polymerase by Karry Mullis of the Cetus Corporation in 1983.
- In this process, the DNA sample is denatured into the separate individual strands.
- Specific DNA primers are used to hybridize to two corresponding nearby sites on opposite DNA strands in such a fashion that the normal enzymatic extension of the active terminal of each primer (that is, the 3' end) leads toward the other primer.
- In this fashion, two new copies of the sequence of interest are generated.

- Repeated denaturation, hybridization, and extension in this fashion produce an exponentially growing number of copies of the DNA of interest.
- The PCR analysis amplified isolated regions on the strands of the DNA under examination.

Restriction fragment length polymorphism...

- RFLP analyzes the length of the strands of the DNA molecules with repeating base pair patterns.
- The basic technique for detecting RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest.
- The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure.

- Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complementary to the probe.
- An RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis.

Lesson No.65

Types of DN fingerprinting methods-2

Random Amplified Polymorphic DNA

- Random Amplified Polymorphic DNA... of PCR reaction, but the segments of DNA
- It is a type that are amplified at random.
- RAPD creates several arbitrary, short primers (8–12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify.
- By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction.
- RAPD does not require any specific knowledge of the DNA sequence of the target organism: the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers' sequence.

ADVANTAGES OF RAPD

- It requires no DNA probes and sequence information for the design of specific primers.
- It involves no blotting or hybridization steps, hence, it is quick, simple and efficient.
- It requires only small amounts of DNA (about 10 ng per reaction) and the procedure can be automated.
- High number of fragments.
- Arbitrary primers are easily purchased.
- Unit costs per assay are low compared to other marker technologies.

DISADVANTAGES OF RAPD...

- Nearly all RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies).
- Co -dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely.
- PCR is an enzymatic reaction, therefore, the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome. Thus, the RAPD technique is notoriously laboratory-dependent and needs carefully developed laboratory protocols to be reproducible.

- Mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret.
- • Lack of a prior knowledge on the identity of the amplification products.
- • Problems with reproducibility (sensitive to changes in the quality of DNA, PCR components and PCR conditions).
- • Problems of co-migration. Gel electrophoresis can separate DNA quantitatively, cannot separate equal-sized fragments qualitatively (i.e. according to base sequence).

Amplified fragment length polymorphism... RFLP

- This technique was also faster than analysis and used PCR to amplify DNA samples. It relied on variable number tandem repeat (VNTR) polymorphisms to distinguish various alleles, which were separated on a polyacrylamide gel.
- By using the PCR analysis to amplify the minisatellite loci of the human cell, this method proved quicker in recovery than the RFLP.
- However, due to the use of gel in its analysis phase, there are issues of bunching of the VTRN's, causing misidentifications in the process.

Lesson No.66

Applications of Fingerprinting

Applications...

- 1) Diagnosis and Developing cures for inherited disorders :
- DNA fingerprinting is used to diagnose inherited disorders in both prenatal and newborn babies in hospitals around the world.
 - These disorders may include cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia, thalassemia, and many others.
 - Early detection of such disorders enables the medical staff to prepare themselves and the parents for proper treatment of the child.
 - In some programs, genetic counselors use DNA fingerprint information to help prospective parents

- In other programs, prospective parents use DNA fingerprint information in their decisions concerning affected pregnancies.
- 2) Biological Evidence to Identify Criminals:
 - Where fingerprints are not available but biological specimens are available like blood or semen stains, hair, or items of clothing at the scene of the crime then these items may prove to be valuable sources of DNA of the criminal.
 - Since the year 1987, innumerable cases have been solved with the help of DNA fingerprint evidence.

- 3) Paternity disputes :
 - Another important use of DNA fingerprints in the court system is to establish paternity in custody and child support litigation. In these applications, DNA fingerprints bring an unprecedented, nearly perfect accuracy to the determination.
- 4) Personal Identification :
 - DNA maybe the best way to identify a person as all body tissues and organs contain the same DNA type. The specimen required also is very small. In fact the US army has been doing DNA fingerprinting of all its soldiers and has a huge databank.