Yr 12 Biology

Module 5: Heredity

Book 6: Inheritance Technologies and Data Analysis

PROFECTUS

Overview

In this topic, the syllabus requires us to be able to analyse the use of technologies to determine inheritance patterns while also analysing data from large-scale collaboratives to identify patterns and relationships.

Dot points covered

- investigate the use of technologies to determine inheritance patterns in a population using, for example: (ACSBL064, ACSBL085) DNA sequencing and profiling (ACSBL086)
- investigate the use of data analysis from a large-scale collaborative project to identify trends, patterns and relationships, for example: (ACSBL064, ACSBL073) the use of population genetics data in conservation management population genetics studies used to determine the inheritance of a disease or disorder population genetics relating to human evolution

1.1 Determining Inheritance Patterns

DNA Sequencing:

DNA sequencing is the process of determining the sequence of nucleotide bases in a piece of DNA. You might have heard of DNA sequencing, for example the human genome was completed in 2003 however sequencing an entire genome remains complex as it requires breaking down the DNA into smaller pieces which can take a large amount of time. There are two main types of sequencing Sanger and next generation sequencing.

Sanger Sequencing:

Sanger sequencing also called the chain termination method was developed by British biochemist Fred Sanger and his colleagues in 1977. Although genomes are now typically sequenced using other methods that are faster and less expensive, Sanger sequencing is still in wide use for the sequencing of individual pieces of DNA, such as fragments used in DNA cloning or generated through polymerase chain reaction (PCR). The ingredients required to perform Sanger sequencing include:

- A DNA polymerase enzyme
- A primer, which is a short piece of single-stranded DNA that binds to the template DNA and acts as a ‘starter’ for the polymerase.
- The four DNA nucleotide (G, A, C, T)
- Template DNA that needs to be sequenced
- Chain terminating versions of all four DNA nucleotides each labelled with a different colour of dye

Method of Sanger Sequencing:

The DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, and DNA nucleotides (dATP, dTTP, dGTP, and dCTP). The four dye-labelled, chain-terminating dideoxy nucleotides are added as well, but in much smaller amounts than the ordinary nucleotides.

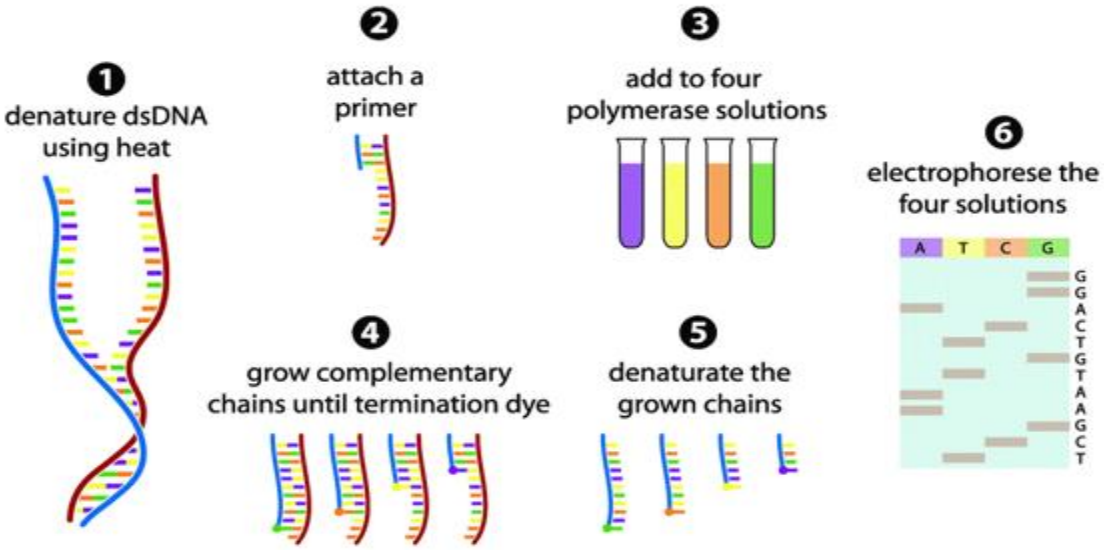
The mixture is first heated to denature the template DNA (separate the strands), then cooled so that the primer can bind to the single-stranded template. Once the primer has bound, the temperature is raised again, allowing DNA polymerase to synthesize new DNA starting from the primer.

DNA polymerase will continue adding nucleotides to the chain until it happens to add a chain terminating nucleotide instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the chain terminating nucleotide.

This process is repeated in a number of cycles. By the time the cycling is complete, it's virtually guaranteed that a chain terminating nucleotide will have been incorporated at every single position of the target DNA in at least one reaction. That is, the tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original DNA. The ends of the fragments will be labelled with dyes that indicate their final nucleotide.

After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called **capillary gel electrophoresis**. Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment crosses the "finish line" at the end of the tube, it's illuminated by a laser, allowing the attached dye to be detected.

The smallest fragment (ending just one nucleotide after the primer) crosses the finish line first, followed by the next-smallest fragment (ending two nucleotides after the primer), and so forth. Thus, from the colours of dyes registered one after another on the detector, the sequence of the original piece of DNA can be built up one nucleotide at a time. The data recorded by the detector consist of a series of peaks in fluorescence intensity, as shown in the **chromatogram** above. The DNA sequence is read from the peaks in the chromatogram.



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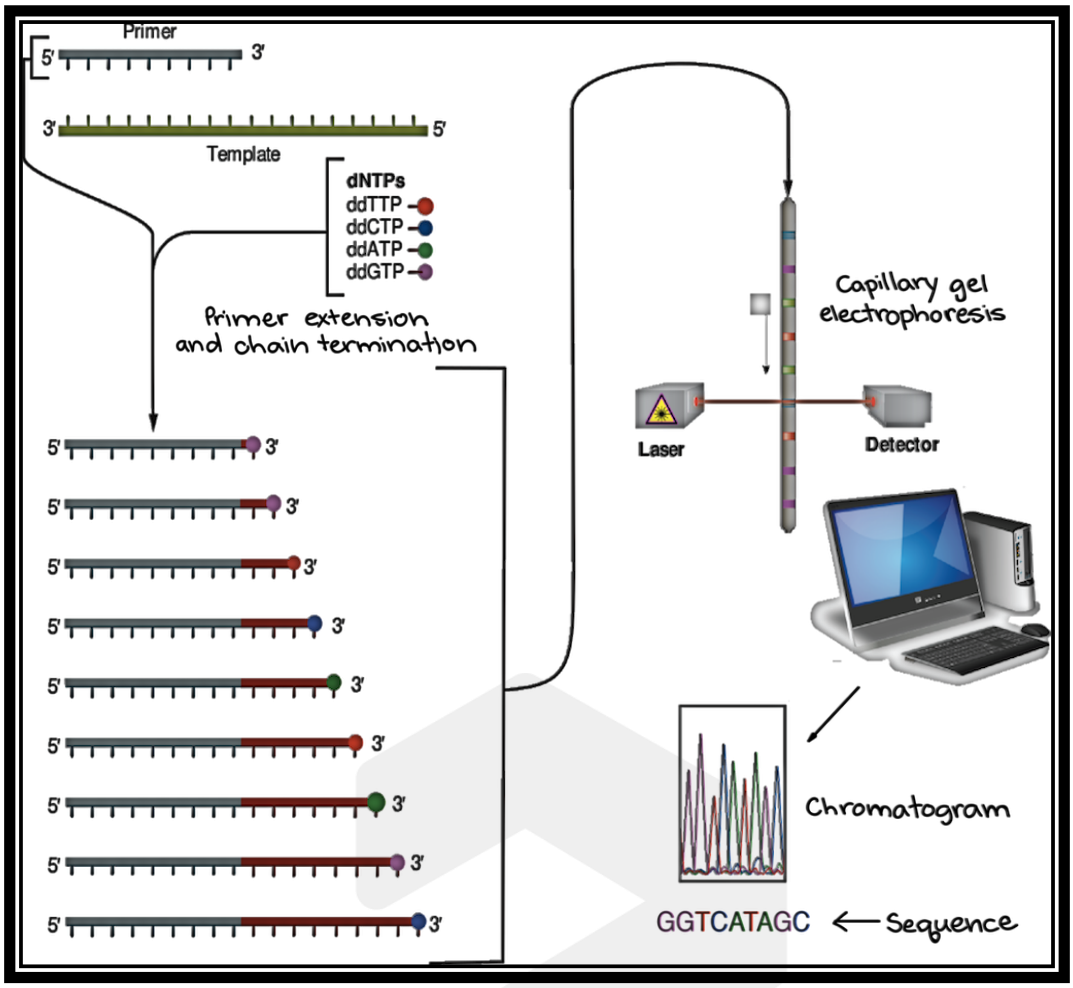


Uses and Limitations:

Sanger sequencing gives high-quality sequence for relatively long stretches of DNA (up to about 900 base pairs). It's typically used to sequence individual pieces of DNA, such as bacterial plasmids or DNA copied in PCR.

However, Sanger sequencing is expensive and inefficient for larger-scale projects, such as the sequencing of an entire genome or metagenome (the “collective genome” of a microbial community). For tasks such as these, new, large-scale sequencing techniques are faster and less expensive.

1.1

Next-Generation Sequencing:

The most recent set of DNA sequencing technologies are collectively referred to as next-generation sequencing. There are a variety of next-generation sequencing techniques that use different technologies. However, most share a common set of features that distinguish them from Sanger sequencing:

- Highly Parallel: Many sequencing reactions take place at the same time
- Micro-Scale: Reactions are tiny and many can be done at once on a chip
- Fast: Because reactions are done in parallel, results are ready much faster
- Low-Cost: Sequencing a genome is cheaper than with Sanger sequencing
- Shorter Length: reads typically range from 50-700 nucleotides in length

Conceptually, next-generation sequencing is kind of like running a very large number of tiny Sanger sequencing reactions in parallel. Thanks to this parallelization and small scale, large quantities of DNA can be sequenced much more quickly and cheaply with next-generation methods than with Sanger sequencing.

Why does fast and inexpensive sequencing matter? The ability to routinely sequence genomes opens new possibilities for biology research and biomedical applications. For example, low-cost sequencing is a step towards personalized medicine – that is, medical treatment tailored to an individual's needs, based on the gene variants in his or her genome.

DNA Profiling:

DNA profiling is the process where a specific DNA pattern, called a profile, is obtained from a person or sample of bodily tissue

Even though we are all unique, most of our DNA is actually identical to other people's DNA. However, specific regions vary highly between people. These regions are called polymorphic. Differences in these variable regions between people are known as polymorphisms. Each of us inherits a unique combination of polymorphisms from our parents. DNA polymorphisms can be analysed to give a DNA profile.

Human DNA profiles can be used to identify the origin of a DNA sample at a crime scene or test for parentage.

1.2

DNA profiling is used to:

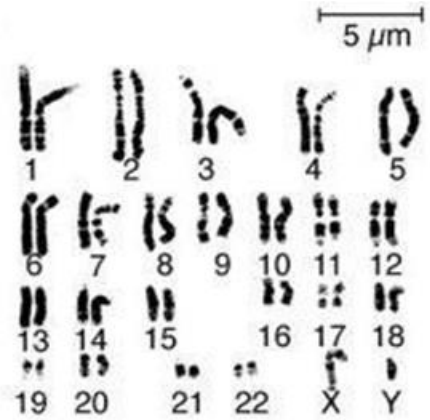
- Identify the probable origin of a body fluid sample associated with a crime or crime scene
- Reveal family relationships
- Identify disaster victims, for example, ESR scientists travelled to Thailand to help identify victims of the 2004 Boxing Day tsunami.

What are short tandem repeats?

One of the current techniques for DNA profiling uses polymorphisms called short tandem repeats. Short tandem repeats (or STRs) are regions of non-coding DNA that contain repeats of the same nucleotide sequence. For example, GATAGATAGATAGATAGATAGATA is an STR where the nucleotide sequence GATA is repeated six times. STRs are found at different places or genetic loci in a person's DNA.

What is a DNA Profile?

One way to produce a DNA profile, is for scientists to examine STRs at 10 or more genetic loci. These genetic loci are usually on different chromosomes. A DNA profile can tell the scientist if the DNA is from a man or woman, and if the sample being tested belongs to a particular person.



1.2

How to Create a DNA Profile using STR:

1. Get a sample of DNA

DNA is found in most cells of the body, including white blood cells, semen, hair roots and body tissue.

Traces of DNA can also be detected in body fluids, such as saliva and perspiration because they also contain epithelial cells. Forensic scientists and Police officers collect samples of DNA from crime scenes. DNA can also be collected directly from a person using a mouth swab (which collects inner cheek cells).

2. Extract the DNA

DNA is contained within the nucleus of cells. Chemicals are added to break open the cells, extract the DNA and isolate it from other cell components.

3. Copy the DNA

Often only small amounts of DNA are available for forensic analysis so the STRs at each genetic locus are copied many times using the polymerase chain reaction (PCR) to get enough DNA to make a profile. Specific primers are used during PCR that attach a fluorescent tag to the copied STRs.

4. Determine the size of the STRs

The size of the STRs at each genetic locus is determined using a genetic analyser. The genetic analyser separates the copied DNA by gel electrophoresis and can detect the fluorescent dye on each STR. This is the same piece of equipment used in the lab for DNA sequencing.

5. Is there a match?

The number of times a nucleotide sequence is repeated in each STR can be calculated from the size of the STRs. A forensic scientist can use this information to determine if a body fluid sample comes from a particular person.

If two DNA profiles from different samples are the same, the chance that the samples came from different people is low. This provides strong evidence that the samples have a common source. To produce a DNA profile, scientists examine STRs at ten, or more, genetic loci. These genetic loci are usually on different chromosomes.

1.2

DNA profiling can be used to compare the differences and similarities between individuals **based on variations in their short tandem repeats (STRs)** in their non-coding region of DNA. This therefore allows the **detection of polymorphisms** at **particular segment of DNA at chromosome locus (genetic marker)** between species in the population. We see the use of DNA profiling to detect polymorphisms in the Albany cycad plant study. Another classic example of the use of DNA profiling is in forensic investigation where the DNA sample from the crime scene is compared against the suspect's DNA. Depending on how closely the DNA bands are, the more likely to the sample is derived from the suspect.

Another example of the use of DNA profiling is in paternity testing. This may be used when there is a lost child and DNA profiling can be used to help confirm the identities of the lost individual that had been spotted after missing in the family for many years. This is when the offspring's DNA is compared against potential fathers' DNA. The more closely of the two individual's DNA band matches with each other, the more closely related they are.

Data Analysis from Large Scale Collaborative Projective

Population Genetics Data:

The primary objective of conservation genetics is to maintain and ensure that the species of conservation concern is able to adapt to changing selective pressures in the environment over time.

Many studies have shown the association between a species' total population having a high genetic similarity being proportional to the species' probability of extinction. This is where population genetics plays a role in conservation management or studies.

1.2

Population genetics focuses on studying genetic diversity of organisms in a population. Information can be gathered regarding how much of variation have been happening within a population of species and measures can be taken to prevent extensive variation and preservation of endangered species. By assuming that there are loci that control the genetic system itself, population genetic models are created to describe:

- The evolution of dominance
- The evolution of sexual reproduction and recombination rates
- The evolution of mutation rates
- The evolution of evolutionary capacitors
- The evolution of costly signalling traits
- The evolution of ageing
- The evolution of co-operation

Use of population genetics for determining inheritance of a disease or disorder:

- Observation of interactions between genes can give idea about what alleles might be dominant and whether disease causing alleles will show dominancy or not.
- Basing on such ideas, whether a disease can be inherited or not can be determined.
- A number of techniques in population genetics are used to measure allelic frequencies in a population, thus giving us an image of what number of alleles are being lost during evolution and what amount are being transferred through gene flow. Analysing these data can give us a rough image of the probabilities of inheriting disorders within a population.

Population Genetics relating to human evolution:

- Questions about human evolution are addressed through analysis of the fossil and archaeological records, combined with analyses of diversity in living human populations.
- Higher genetic diversity in Africa has been said to indicate an origin in Africa, but in fact, the characteristic pattern of this diversity indicates only a larger number of ancestors, not greater time-depth, within Africa.

1.

- The oldest fossils showing modern human characteristics have been found in Africa and date to about 130,000 years ago. The oldest related modern human fossils outside of Africa appear in the Middle East, dating from about 90,000 years ago.
- An alternative view is that humans have been evolving within a single evolutionary population, which, though structured, has been prevented from divergence into a new species within the last million years by gene flow.
- Larger genetic distances between populations in sub-Saharan Africa and those in Europe or Asia generate ideas that people have migrated out of Africa and thus, the genetic variations in populations have split globally.
- However, variable rates of genetic drift and gene flow between continental regions are a more likely explanation for observed geographic patterns of diversity.
- The main point is that genetic diversity data can be interpreted to fit either model for modern human origins, and therefore have not resolved the debate.

Questions

1) Explain DNA sequencing and its purpose. 3 marks
2) Describe the method of Sanger sequencing. 6 marks
3) Why is Sanger-sequencing being replaced by next-gen sequencing. 3 marks
4) Explain DNA profiling and its purpose. 3 marks
5) What are short tandem repeats? 2 marks
6) Describe the method of DNA profiling. 5 marks
7) How can DNA profiling be used to determine inheritance patterns? 4 marks
8) Explain how population genetics can be used to determine inheritance patterns. 7 marks

Model Answers:

1) DNA sequencing is the process of determining the sequence of nucleotide bases in a piece of DNA. DNA sequencing is extremely important as it allows scientists to determine the genetic information carried by the DNA and how this information is translated into structures and functions of the body. It allows scientists to determine changes in genes, associations with diseases and identify any problems occurring in the genome.

This question is worth 3 marks. 1 mark for the definition of DNA sequencing, two marks for explaining its purpose and giving an example.

2) In Sanger sequencing, the DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, DNA nucleotides and four dye-labelled, chain-terminating dideoxy nucleotides in much smaller amounts than the ordinary nucleotides. The mixture is heated to separate the strands and then cooled to allow the primer to bind to the sample. It is then heated again allowing DNA polymerase to synthesise new DNA from the primer until a chain terminating nucleotide is coded for. This process is repeated a number of times, guaranteeing a chain terminating nucleotide to be incorporated at every single position of the target DNA in at least one reaction. The tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original DNA. The ends of the fragments will be labelled with dyes that indicate their final nucleotide.

   After this, the strands go through gel electrophoresis where they are put through a gel matrix. Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment crosses the “finish line” at the end of the tube, it’s illuminated by a laser, allowing the attached dye to be detected. This allows for a recreation of the original DNA and this information can be fed to a chromatogram from which the DNA sequence can be read as fluorescent peaks.

This question is worth 6 marks. 1 mark for listing all the ingredients that go into the process. 1 mark for denaturing and cooling. 2 marks for explaining how repeating the cycle can lead to a DNA sequence being created. 1 mark for explaining gel electrophoresis. 1 mark for describing how the sequence is read through the chromatogram.

3) Sanger sequencing is being replaced by next-gen sequencing due to it's rising popularity and ease of use. There are many advantages that next-gen sequencing provides such as reactions being tiny and many being able to take place at the same time increasing efficiency. Faster results due to reactions being done parallel to one another and cheaper cost compared to Sanger sequencing.

This question is worth 3 marks. 1 mark for explaining why next-gen is on the rise. 2 marks for giving examples of why it is being used more often.

4) DNA profiling is the process where a specific DNA pattern, called a profile, is obtained from a person or sample of bodily tissue. DNA profiling has many purposes such as helping identify origins of fluids and tissue sample for a crime scene investigation, helping link family relationships of people and allowing the identification of disaster victims with the help of DNA profiles of their relatives.

This question is worth 3 marks. 1 mark for definition of DNA profiling. 2 marks for giving examples of how DNA profiling can be used.

5) Short tandem repeats (or STRs) are regions of non-coding DNA that contain repeats of the same nucleotide sequence. For example, GATAGATAGATAGATAGATAGATA is an STR where the nucleotide sequence GATA is repeated six times.

This question is an easy 2 marker. 1 mark for definition. 1 mark for an example of a STR

6) There are five main steps in the process of creating a DNA profile. It starts with DNA samples being collected so they can be analysed and their genetic information revealed. DNA is contained within the nucleus of cells. Chemicals are added to break open the cells, extract the DNA and isolate it from other cell components. STRs found within the DNA are copied over numerous times using polymerase chain reactions (PCR) a certain primer is also added during this process which attached fluorescent dye to the copied STRs. The DNA is separated with gel electrophoresis and the dye is used to measure the size of the STRs. The number of times a nucleotide sequence is repeated in each STR can be calculated from the size of the STRs. A forensic scientist can use this information to determine if a body fluid sample comes from a particular person.

This question is worth 5 marks. 1 mark for explaining each part of the process (getting a sample of DNA, extracting the DNA, copying the DNA, determining size of STRs and finding a match)

7) DNA profiling is the process where a specific DNA pattern, called a profile, is obtained from a person or sample of bodily tissue. DNA profiling can be used to determine inheritance patterns in a multitude of ways. DNA profiling can be used to compare the differences and similarities between individuals based on variations in their short tandem repeats (STRs) in their non-coding region of DNA. This therefore allows the detection of polymorphisms at particular segment of DNA at chromosome locus (genetic marker) between species in the population. Another example of the use of DNA profiling is in paternity testing. This may be used when there is a lost child and DNA profiling can be used to help confirm the identities of the lost individual that had been spotted after missing in the family for many years. This is when the offspring's DNA is compared against potential fathers' DNA. The more closely of the two individual's DNA band matches with each other, the more closely related they are.

This question is worth 4 marks. 1 mark for definition of DNA profiling. 3 marks for examples given that show how DNA profiling can be used and how the inheritance patterns can be determined.

8) Population genetics assesses the differences and similarities between the genetic makeup of ancient and modern populations in order to model the evolutionary and migratory paths of modern cultures. Many studies have shown the association between a species' total population having a high genetic similarity being proportional to the species' probability of extinction. This is where population genetics plays a role in conservation management or studies. Population genetics focuses on studying genetic diversity of organisms in a population. Information can be gathered regarding how much of variation have been happening within a population of species and measures can be taken to prevent extensive variation and preservation of endangered species. Population genetic models can be created from this information which describe inheritance patterns such as the evolution of sexual reproduction and recombination rates.

Observation of interactions between genes can give idea about what alleles might be dominant and whether disease causing alleles will show dominancy or not. Basing on such ideas, whether a disease can be inherited or not can be determined. A number of techniques in population genetics are used to measure allelic frequencies in a population, thus giving us an image of what number of alleles are being lost during evolution and what amount are being transferred through gene flow. Analysing these data can give us a rough image of the probabilities of inheriting disorders within a population. This allows us to determine inheritance patterns within a population easily identifying how certain diseases are passed from one generation to the next.

The Human Genome Project (1990-2003) was an extremely large-scale collaborative project which sought to identify the position of all the genes in the human chromosome and create a database for that information for public use. It gave scientists a much clearer understanding of the causes of genetic disorders as well as the patterns of evolution. The HGP allowed us to compare our modern genome with the genetic makeup of ancient common ancestors and to see why similar species such as Homo neanderthalensis (Neanderthals) diverged. It also accounted for slight genetic differences that have appeared in different cultures in our more recent history.

This question is worth 7 marks. 1 mark for explanation of population genetics. 2 marks for describing how population genetics can figure out if certain species are more likely to go extinct allowing for extra emphasis being put on conservation strategies. 2 marks to describe how population genetics can predict the transfer of certain diseases and disorders from one generation to the next. 2 marks to give an example of how population genetics has been used in present times, and how it has benefited scientific research (HUMAN GENOME PROJECT).