Module 2 : DNA Technology

In this module, a process of identification of a person from his DNA is illustrated by some videos and animations.

You will have better understanding on the key techniques of DNA fingerprinting, including :

- Isolation of DNA
- Amplification of DNA
- Gel Electrophoresis
- Determination of DNA concentration and its purity.

2a. What is DNA?

Deoxyribonucleic acid (DNA) is the hereditary molecule in all cellular life-forms and some viruses. It is a double-stranded helix structure. Each helix is made up of nucleotides with different bases, namely Adenine, Thymine, Cytosine and Guanine. The sequence of the nucleotides determines the genetic code that codes for different genetic characteristics of living organisms. Every person has unique DNA sequence gives one's unique identity. Therefore, DNA technology is a very valuable tool for disease diagnosis and forensic investigation nowadays.

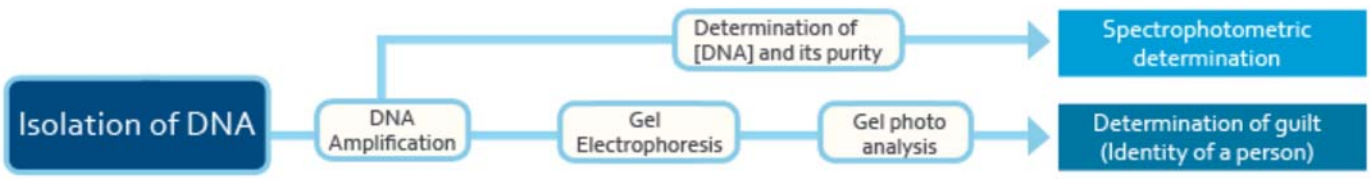
In this module, we will learn the DNA technology and its application with the help of a case study.

2b. Case study: DNA fingerprinting

In this case study, the ultimate goal of a series of experiments is to determine one's identity in order to find out identity of a person. First of all, samples of cheek cells are to be obtained using sterile buccal swab brushes. Then, DNA is to be isolated from the cheek cells samples.

After DNA isolation, the small amount of DNA needs to be amplified. After DNA amplification process, agarose gel electrophoresis is to be carried out, and find out the identify of a person, e.g. a guilt/ family members by the gel photo analysis. On the other hand, the concentration and the purity of DNA can be determined by spectrophotometric determination or Ethidium Bromide fluorescent determination.

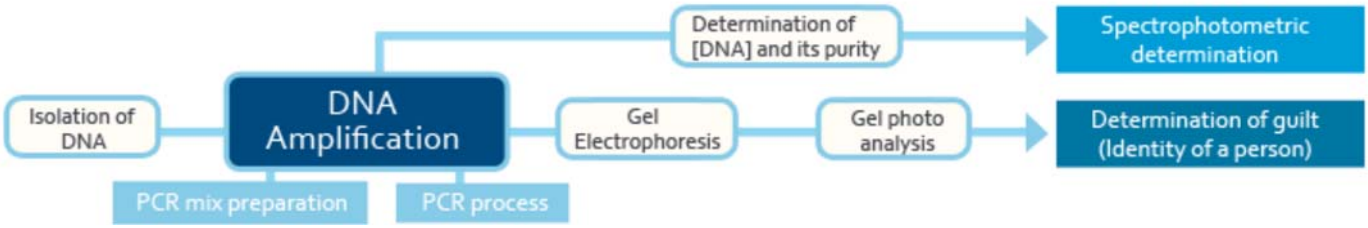
2b(i). Isolation of DNA



DNA extraction requires heat treatment to lyse the epithelial cells and to degrade compounds inhibitory to amplification. After the heat treatment, centrifugation needs to be carried out to pellet debris in order to obtain around 0.5 to 3µg of DNA from each buccal sample.

Video : Isolation of DNA (see website)

2b(ii). DNA Amplification by PCR



The amount of cells collected from patients or suspects in disease diagnosis or forensic investigation may not be enough for DNA analysis.

Therefore, the DNA isolated from the collected cells needs to be amplified by polymerase chain reaction carried out in the PCR machine.

PCR mix preparation

PCR mixture includes
dd H_2O
10X buffer
dNTPs
Primer mix
DNA sample
Taq DNA polymerase

Video: PCR mix preparation (see website)

PCR process

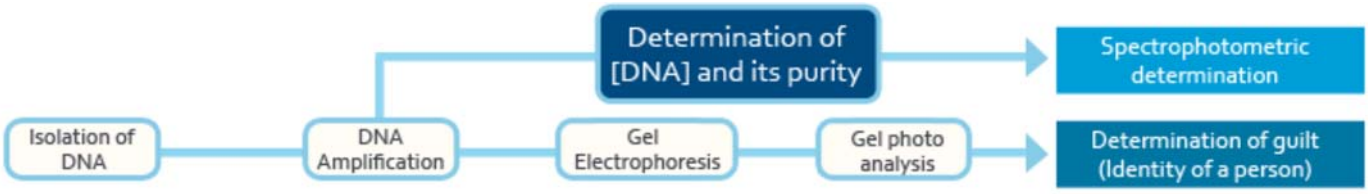
In a PCR cycle, 3 steps taken place at different temperature are involved. The first step involves the denaturation of the double-stranded DNA, by disrupting the hydrogen bonds between complementary bases at high temperature, 94℃ for 15 seconds.

After denatured into single-strand DNA, annealing of primers with sequences that closely match with the template sequences occurred at 55℃ for 15 seconds, forming the primer-template hybrids. The DNA polymerase binds to this hybrid for DNA synthesis to take place. The final step of each cycle is carried out at 72℃ for 45 seconds.

In this period, the free dNTPs complementary to the templates are added in 5' to 3' direction to synthesize new DNA strands. Typically, amplification of DNA by PCR consists of 20-30 repeated cycles of the above temperature change.

Animation: PCR process (see website)

2b(iii). Determination of concentration and purity of nucleic acids



Concentration of DNA

Spectrophotometric assessment of nucleic acid concentration is based on the strong absorption of light at 260nm of DNA. The absorption is measured at the peak of the nucleic acid spectra and concentrations can be calculated using the equations as follows:

dsDNA: 50/(1/OD_{260} of the DNA sample) = concentration of DNA sample (µg/ml)

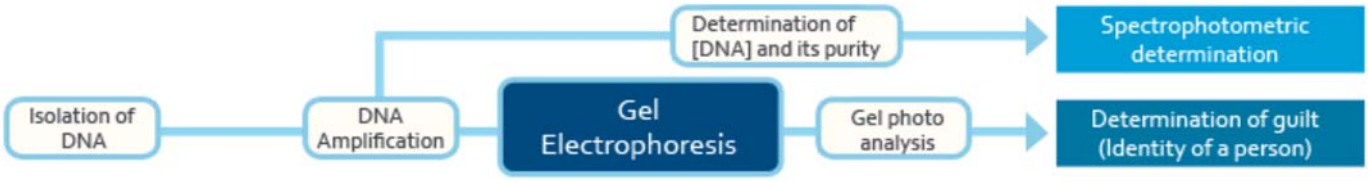
ssDNA: 40/(1/OD_{260} of the DNA sample) = concentration of ssDNA sample (µg/ml)

Purity of DNA

The ratio of absorbance at 260 nm and 280 nm, [A_{260} / A_{280}] provides an estimate of the purity of the nucleic acid. A pure sample of DNA should have a ratio of 1.8-2.0. For sample contaminated with protein, the ratio should be lower.

Video: Spectrophotometric determination of DNA concentration (see video)

2b(iv). Gel electrophoresis



After amplification of the DNA extracted from buccal cells of different individuals, gel electrophoresis of different DNA samples can be carried out. Gel electrophoresis involves the separation of DNA, according to their size, via their migration through a gel under the presence of an electric field. Under the influence of the electric field, negatively charged DNA will migrate towards the positive charged end of the gel.

DNA samples with different sequences show particular band patterns on the gel photo which are fluorescence images captured under UV transillumination. Separation of DNA is usually done by agarose gel electrophoresis.

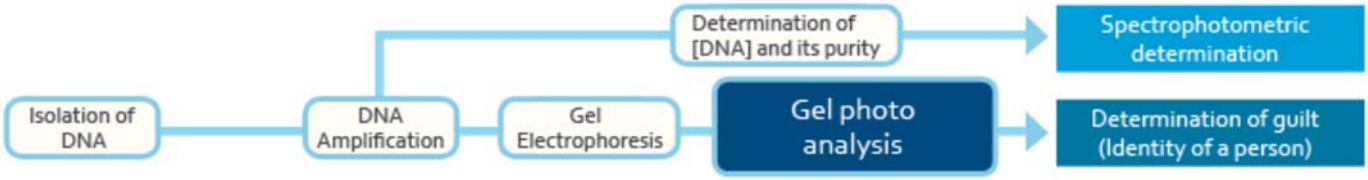
Agarose gel electrophoresis

Agarose gel contains microscopic pores that allow it to act as a molecular sieve to separate DNA by size. When samples of DNA are loaded into the wells, they will migrate through the gel towards the

positive electrode since DNA has a strong negative charge at neutral pH. The smaller fragments migrate through the gel faster than the larger fragments. A ladder, or known standard, is run at the same time as the DNA analyzed as a reference for size estimation.

Video : Agarose gel electrophoresis (see website)

2b(v). Gel photo analysis



When electrophoresis completes, the Ethidium Bromide (EtBr) treated gel is exposed to UV light. A property of EtBr is that it fluoresces under UV light when intercalated with DNA. Therefore, the results are visualized, in which the DNA will appear as bands along the agarose gel. The position of the band corresponds to the relative size of the DNA, and can be estimated by referencing known standards.

Here's the gel photo obtained by the above experiment.