Analytical Chemistry

Electrophoresis and DNA Fingerprinting

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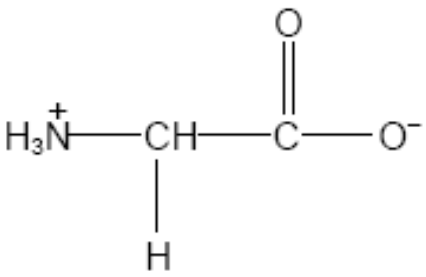
Electrophoresis

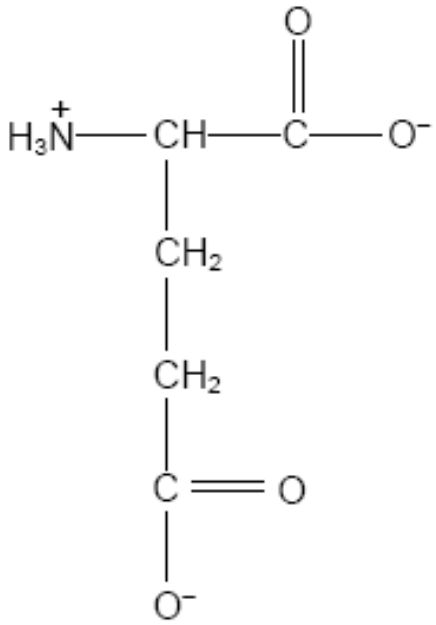
- Sample : **Amino acids or peptides, nucleic acids, proteins.**
- **Separation of ions placed in an electric field.**

- **Buffer solution carries the ions along the agarose gel**

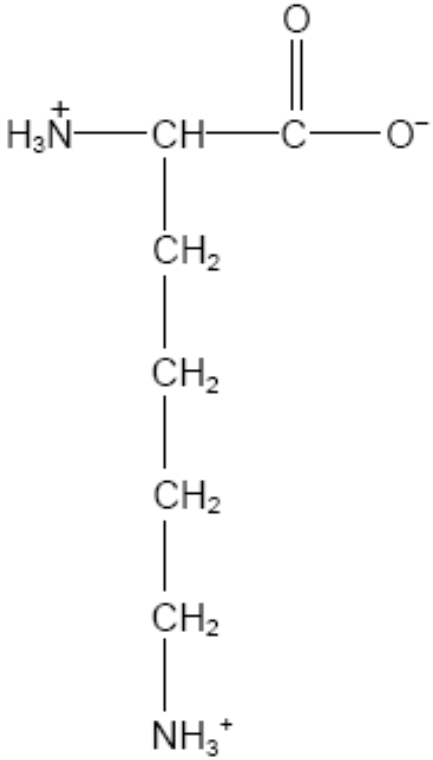
Electrophoresis



glycine



glutamic acid



lysine

Figure 2.37 – structures of glycine, glutamic acid and lysine at pH 7

Electrophoresis

The **original spot** will have been **separated** into three spots, as in Figure 2.38.

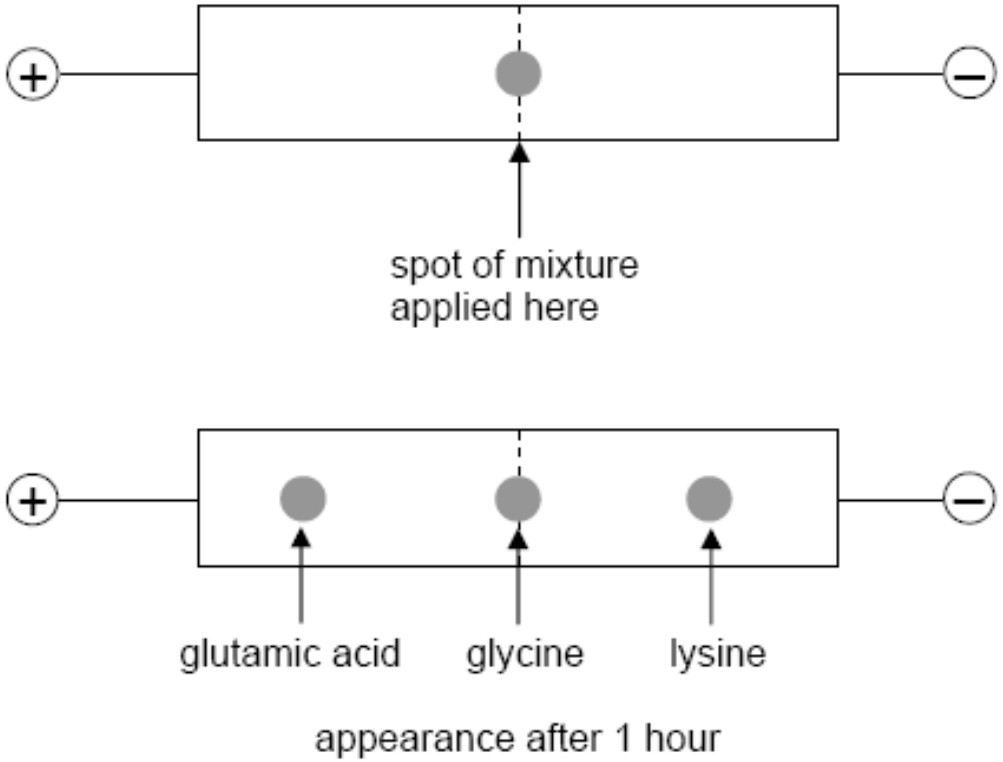


Figure 2.38 – results of electrophoresis of glycine, glutamic acid and lysine at pH 7

Electrophoresis

- Gel electrophoresis is applied to :
  - measure the **relative masses** of macromolecules;
  - **prepare nucleic acids and polypeptides for sequencing the component monomers** e.g. purine and pyrimidine bases and amino acids; and
  - **separate proteins**, so antibodies can be raised.

Electrophoresis

- The main components used in a gel electrophoresis analysis :
  - an electrophoresis **chamber**;
  - a **gel support** medium soaked in conducting **buffer**;
  - a means of generating an **electric field** –*e.g.* a **power pack**;
  - **probes for detecting** and/or measuring the separated molecules; and, if necessary,
  - a **means of extracting** the individual products.

Electrophoresis

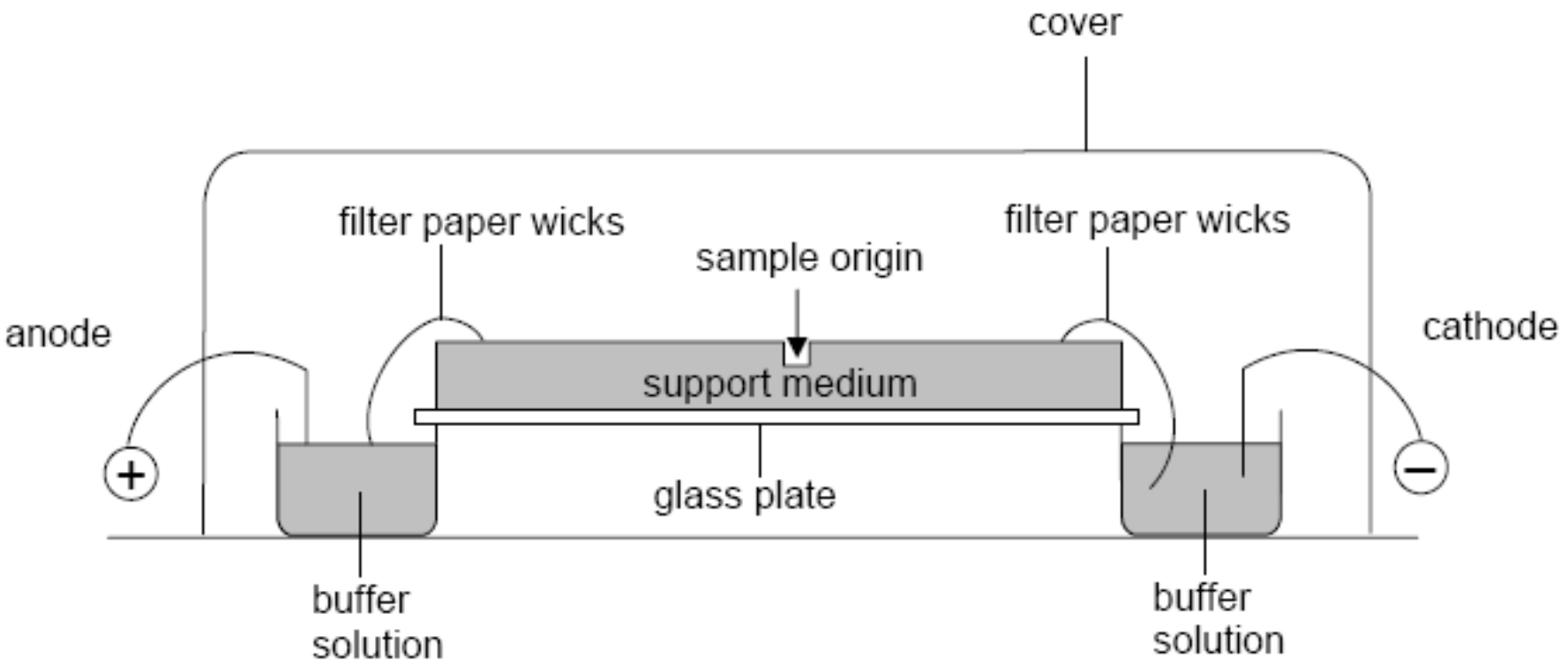


Figure 2.39 – gel electrophoresis apparatus

Electrophoresis

- **Sample mixture** is **placed in a gel.**
- **Separated into its constituents by applying an electric field to the gel**, which is **soaked in a liquid buffer**.
- **Components** in the sample mixture **is charged.**
- **Nucleic acids (DNA)** are **usually negatively charged (at the phosphate groups)** at the pH used for their separation using electrophoresis.

Electrophoresis

- The **molecules move in response to an electrical field applied across the mixture**.
- The rate of progress of the molecules depends on their **size, charge** and **shape**.
- **On separation**, the **components** are **concentrated into bands or zones**.

Electrophoresis

- Factors affecting separation of components :

  - **Voltage**: the **velocity of a molecule is directly proportional to the voltage gradient** across the gel.
  - **Size**: **smaller molecules migrate quicker** than larger molecules carrying the same charge.
  - **Shape**: a molecule with **lots of side-chains experiences more frictional resistance**
  - **Buffer pH**: The extent, and direction, of **ionisation depends on the pH of the buffer**.
  - **Temperature**: a **rise in temperature can speed up** electrophoresis

Electrophoresis

- Applications:
  - checking the adulteration of foods,
  - chromosome sequencing,
  - DNA fingerprinting and
  - characterising the chemicals responsible for allergic symptoms.
  - screen infants' milk for α-lactoglobulin, a protein which is lethal for small babies.

Some medical applications of gel electrophoresis

- ***Defects in newborn babies.*** Neural tube malformations can be detected from **proteins leaking from the central nervous system of a foetus into the amniotic fluid**. Analysing these proteins can **indicate spinal problems in new-born babies**.

- ***Alcohol abuse.*** Can be investigated by analysing blood. **Excess alcohol is associated with changes in acidic proteins and glycoproteins in blood plasma**.

- *'Fish eye' disease*. Protein samples from people with this condition have been analysed, suggesting that there is a deficiency in an enzyme system associated with lipid metabolism.

Some medical applications of gel electrophoresis

- *Heart attacks*. Identify more sensitive 'marker' proteins which will indicate early blockage in blood vessels.

- *Assessing fitness*. serious increase in the amount of protein in urine, which can be detected by two-dimensional electrophoresis.

Genetic fingerprinting

- **10% *of the DNA comprises of the* genes –** (povides the genetic blueprints for making proteins) - and will be **almost identical in most members of a particular species**.
- The **other 90% contain sequences of bases** (**about 10-50 base pairs in length**) that are **repeated several times** – minisatellites or **VNTRs** (variable number of tandem repeats)
- Minisatellites are **unique** for each individual, except for **identical twins.**
- **Members of a family** throughout many generations will **have similar minisatellite patterns** to each other.

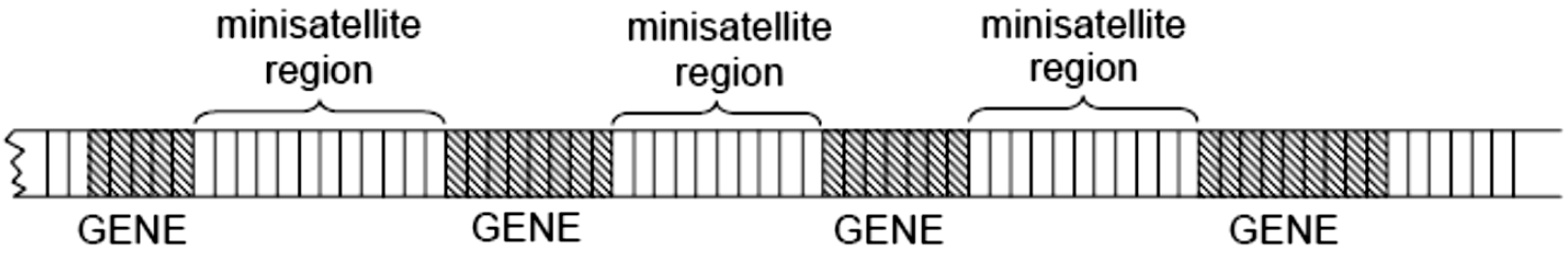


Figure 2.42 – strand of DNA showing genes and minisatellites

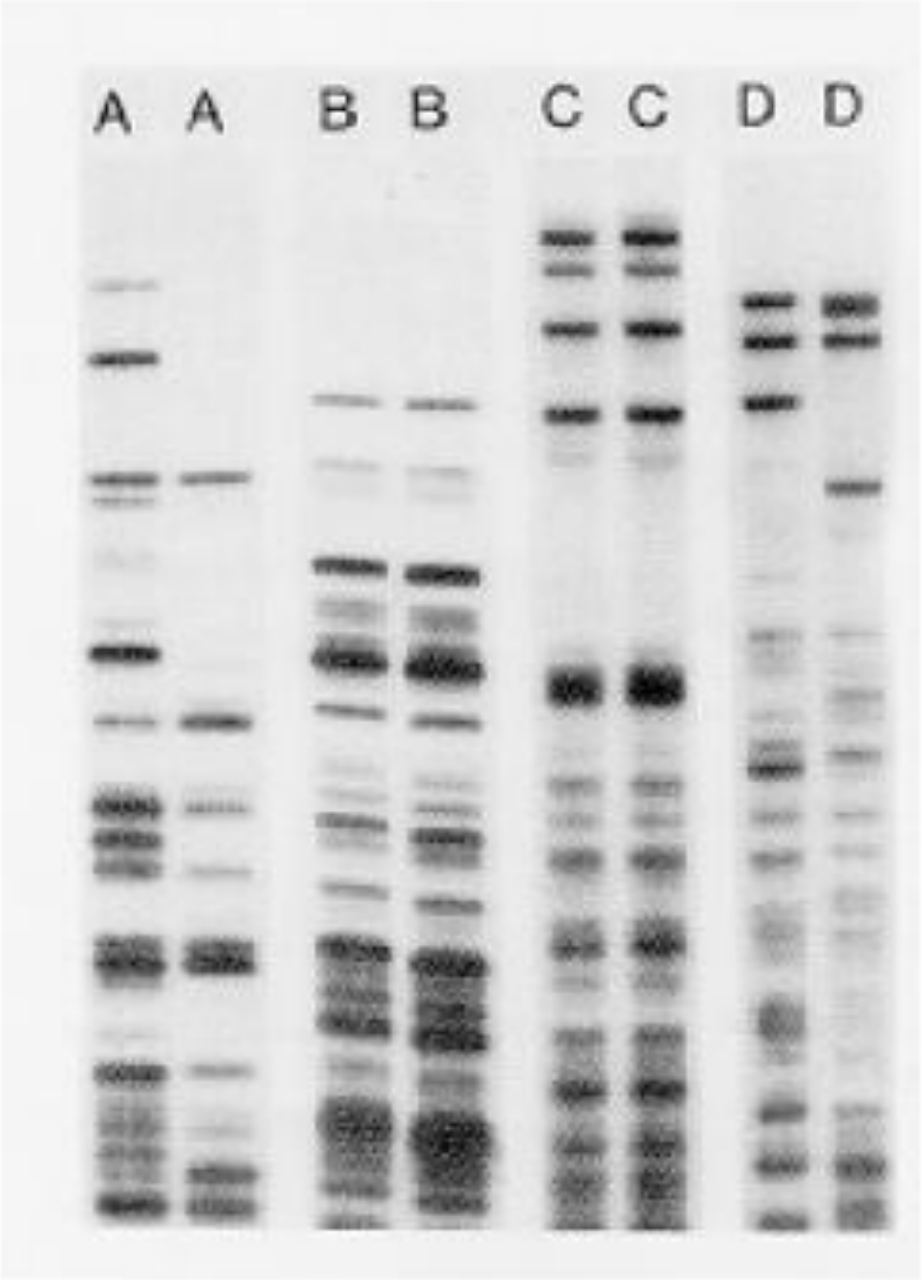


Figure 2.43 – DNA from twins

Technique

1. E**xtraction of the DNA** from sample such as **blood**, **hair**, **inner cheek cells**, **semen** or **skin**.

2. **DNA is then broken into fragments** by a **restriction enzyme** where it cuts the DNA molecule at specific sites.

4. Fragments produce ➔ separated by **electrophoresis**.

Since the **phosphate groups of DNA are negatively charged**, all **DNA samples will move towards the anode**, but the **smaller fragments will move faster** than the larger ones.

5. **DNA bad pattern** (invisible to naked eye) are **transferred onto a nylon membrane**

Technique

6. ^{32}P labeled DNA probe binds to the bands of DNA.

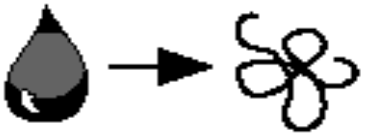
7. **X-ray sensitive film is placed over the plate**, it will **record the pattern originally on the gel plate**

8. Alternative way : use probe that makes bands fluoresce in UV light.

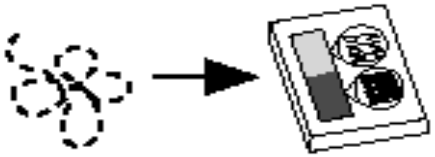
http://www.youtube.com/watch?v=5eMsgidAY5E

THE PROCESS OF DNAFINGERPRINTING

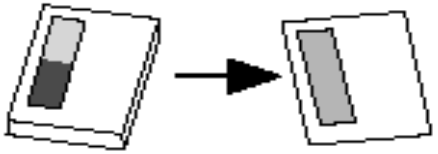
1. The process begins with a blood or cell sample from which the DNA is extracted.



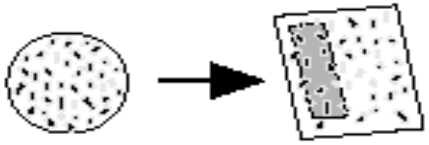
2. The DNA is cut into fragments using a restriction enzyme. The fragments are then separated into bands by electrophoresis through an agarose gel.



3. The DNA band pattern is transferred to a nylon membrane.



4. A radioactive DNA probe is introduced. The DNA probe binds to specific DNA sequences on the nylon membrane.



5. The excess probe material is washed away leaving the unique DNA band pattern.

6. The radioactive DNA pattern is transferred to X-ray film by direct exposure. When developed, the resultant visible pattern is the DNA FINGERPRINT.

Technique

- **Polymerase chain reaction (PCR)** can be used **when the amount of available DNA is smaller**,
- After the DNA has been broken up into fragments using restriction enzymes, **an individual segment is extracted.**
- **Heating to separates the two DNA strands from each other.**
- The **sample** is **cooled** and a **short "primer" length of DNA which matches** the end of the segment is **added, together with the enzyme DNA polymerase** and the **four monomer nucleotides** A, G, C, T.
- **On warming**, the **bases** will **bind** together.

Technique

- The **process is repeated many times** until a sufficient amount of DNA has been made.
- http://www.youtube.com/watch?v=rgj0BifStM8

- **Samples of DNA to be analysed** are **placed in wells near the cathode** of an agarose gel plate.

The uses of genetic fingerprinting

- **paternity testing**
- **establishing other familial relationships** – between both the living and the dead
- establishing the relationship between **archaeological** artifacts
- **forensic testing**
- **Medical applications** - investigations of whether cases of tuberculosis or cancers are re-infections from previous illnesses, or brand new infections.

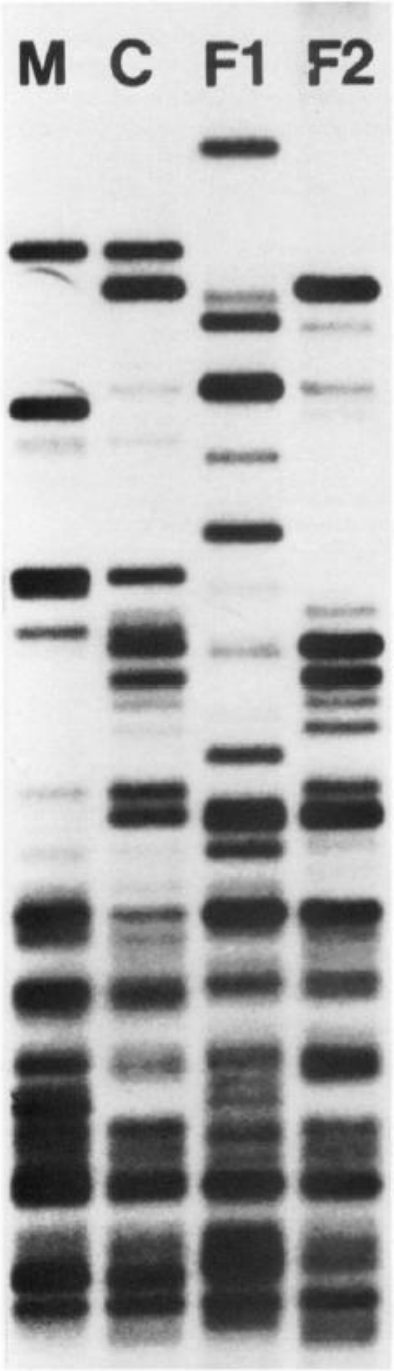


Figure 2.44 – genetic fingerprints of a child, C, and its mother, M, and two possible fathers, F1 and F2

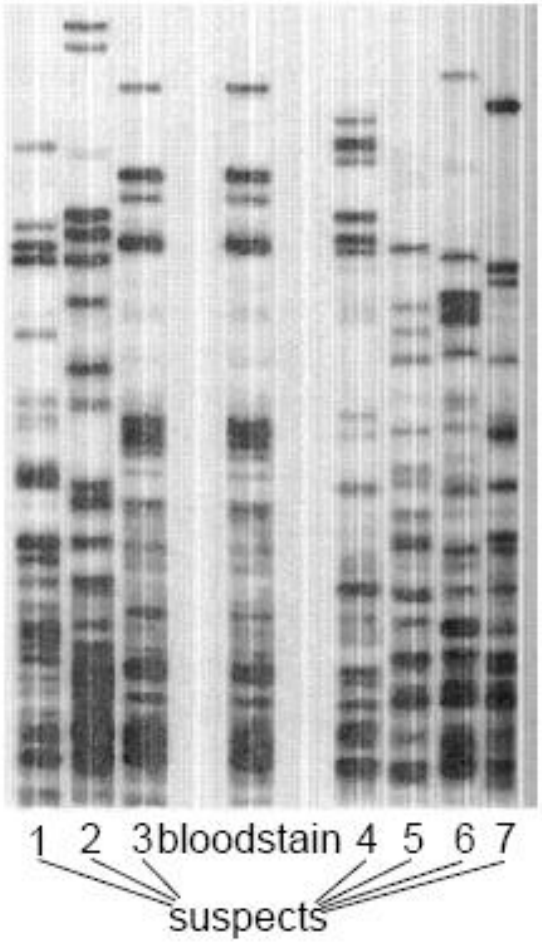


Figure 2.45 – DNA fingerprints of seven suspects and blood taken from the scene of a crime