

Labotratory Resources



Department of Botany

PANSKURA BANAMALI COLLEGE
(AUTONOMOUS)

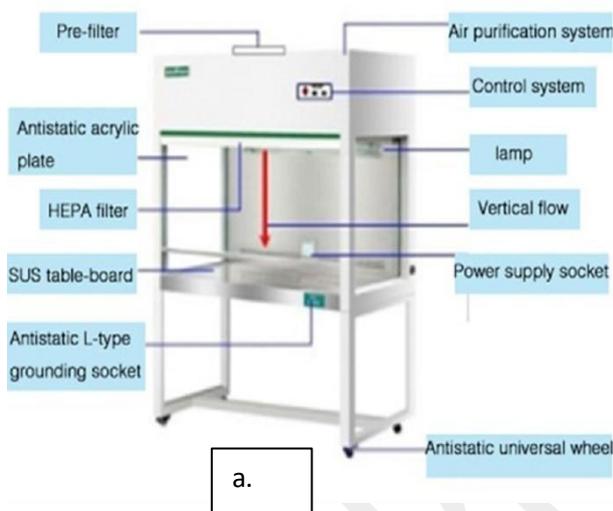
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7. To separate proteins using PAGE.
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a. Laminar Air Flow

Key Characteristics:

- A laminar airflow system aims to reduce turbulence and maintain uniformity in the flow to reduce contamination.
- A laminar flow hood consists of a filter pad, a fan and a HEPA (High Efficiency Particulates Air) filter. which removes all airborne contamination to maintain sterile conditions.
- Working platform and Chamber for aseptic culture with UV C light



PrimeSurgicals Vertical Wing Nut Stainless Steel Double Walled Autoclave



b. Autoclave

- **Pressure Cooker Type/Laboratory Bench Autoclaves (N-type):** This autoclave is commonly used around the world for moist heat sterilization. Autoclaves typically yield a temperature of about 121 degrees Celsius at 15 pound/square inch pressure taking about 15-20 minutes to complete the sterilisation process.
- It contains an air and steam discharge tap, a safety valve, and a pressure gauge. It also contains an electric immersion heater located at the bottom of the chamber.
- Place the instruments inside the chamber. Close the lid and tighten the screws then switch on the electric heater for sterilization of glass goods and instruments

c. Hot Air Oven

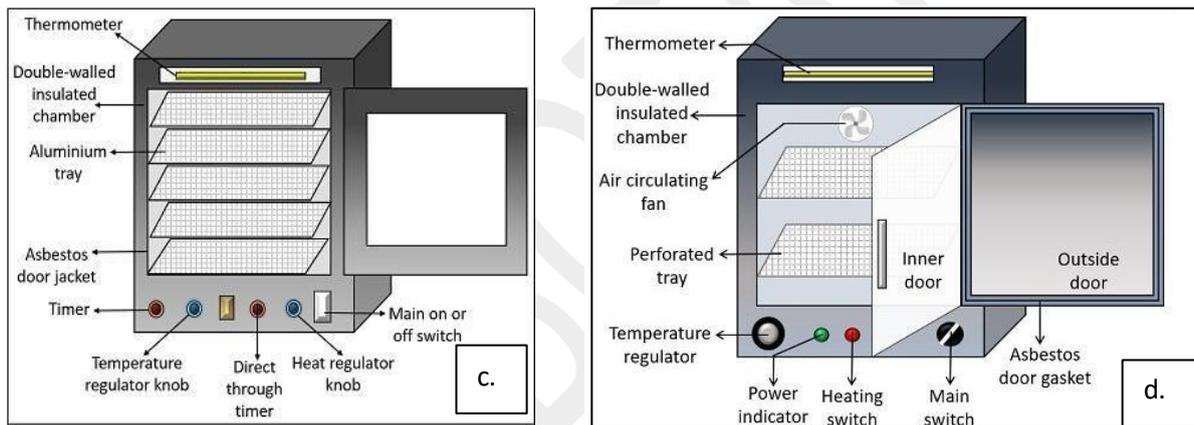
A hot air oven is a type of **dry heat sterilization** that works on the principle of **heat conduction**, in which the articles are sterilized layer by layer, starting from the surface towards the centre. In this system, dry heat is recirculated within a chamber at a temperature ranging between 50-300 °C to sterilize **thermally stable objects**. A hot air oven makes the use of dry heat to **oxidize** the **cellular components** of the microorganisms and their spores to kill them.

1. Exterior Components

- **Main switch, Temperature regulator, Thermostat and Asbestos door jacket:**

2. Interior Components

- **Inner chamber, Temperature sensor, Air vents, Aluminium trays, Circulating fans and Heating element**



d. Bacteriological incubator

The working of a bacteriological incubator depends upon **thermo-electricity**. A thermostat at the incubator's top regulates a desirable temperature within the chamber.

The bacteriological incubators work by using a heating system only. Due to this reason, we call such incubators "**heated incubators**".

The term "**Bacteriological incubator**" itself clears that it only incubates bacteria.

- It maintains a desirable temperature range of 10°C above ambient to 60°C.
- Most bacteria grow best at a temperature of 37 °C.

The bacterial growth within the bacteriological incubator is **independent** of the **external environment**. In general, the incubator utilizes heating and no-heating cycle.

It is almost like hot air oven.

e. -20 C deep freezer

Deep freezers are the testing equipment that are used to **preserve and store food products, medical equipment, blood samples, medicines and injections, etc.** for a long period of time. Deep Freezers are used for industrial purposes as well as for household purposes. In chest type deep freezer the door is opening horizontally.

f. Orbital Shaker

- An orbital shaker works by generating a circular shaking motion at a slow speed of 25-500 rpm.
- The shaker contains an oscillating board with spring attached clips.
- The holder that hold the vessels as the device shakes to blend, agitate, or mix the substances in the vessels.



g. Laboratory Hot Plate

laboratory hot plates in both rectangular and round shapes with surface temperature range up to 350°C and optional 500°C. Heating surface is made of either MS, Stainless Steel or Cast Iron. Temperature is controlled through energy regulator or digital PID controller which efficiently controls temperature and also displays set value and process value.

H. Cyclomixers / Vortex mixers

- Designed for mixing liquids in Laboratories
- Speed Regulation through knob provided on the control panel
- Interchangeable mixing heads for use with variety of tubes
- Touch/ Continuous Operation mode Selection through bi-directional Switch

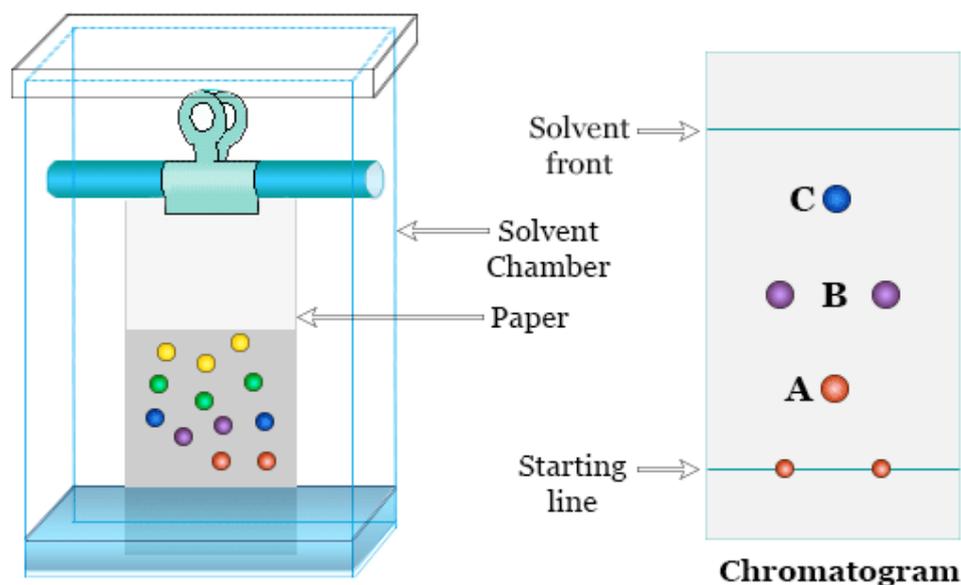


I.

- Electronic balance
- Ph meter
- Hot Water bath



PAPER CHROMATOGRAPHY



Priyamstudycentre.com

Procedure

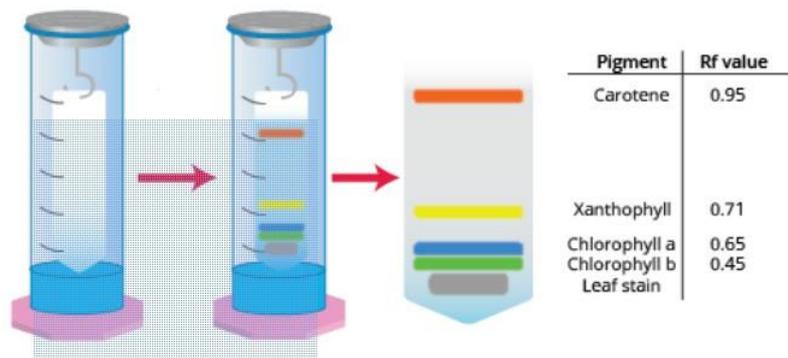
- In this experiment, spinach leaves are used to separate different pigments.
- Pick a few fresh and green leaves of spinach and wash it.
- Cut out small pieces of spinach using scissors. Add them to the mortar.
- Accurately measure 5ml acetone using a measuring cylinder and add it into the mortar.
- With the help of mortar and pestle, grind the spinach leaves into a smooth paste.
- Shift the prepared paste of spinach into the watch glass with the help of a spatula.
- Place a filter paper strip with a tapering notch towards one ending of the strip.
- Horizontally trace a line with a scale and a pencil that is 2 to 3 cm apart from the notch's tip.
- Using a capillary tube, add 1 drop of the extract of the pigment in the midsection of the line.
- Let the drop dry. Repeat the same process of adding a drop and allowing it to dry for 4-5 times.
- In the chromatographic chamber, pour the ether acetone solvent.
- Make sure to folded and stapled an end side of the paper.

- Suspend the strip in the chamber.
- The loading spot remains about 1 cm above the level of the solvent.
- Let the chamber remain uninterrupted for a while.
- We can notice that the solvent passes along the paper scattering various pigments of the blend to different distances.
- Once the solvent reaches 3/4th of the strip, carefully take the strip off.
- Allow the strip to dry.

Observation/ Result

The dried paper strip displays four different bands. Discrete pigments can be distinguished with the help of colours.

The pigment's movement rate is measured by the R_f (retention factor) value. R_f value = distance transported by pigment from origin to centre of pigment spot/distance from the origin to the solvent front. By applying this formula, you can determine the R_f value.



Conclusion

1. The Carotene pigment is observed at the topmost as an orange-yellow band of pigments distinctively.
2. Just below this band, a yellowish band appears which indicates the pigment xanthophyll.
3. The third band appearing dark green/blue indicates chlorophyll-a pigment.
4. The yellowish-green band present at the bottom is the chlorophyll b pigment.

Exp 2: Experiment on amino acids

A paper chromatography experiment is used for the identification or separation of individual amino acids from a mixture of amino acids.

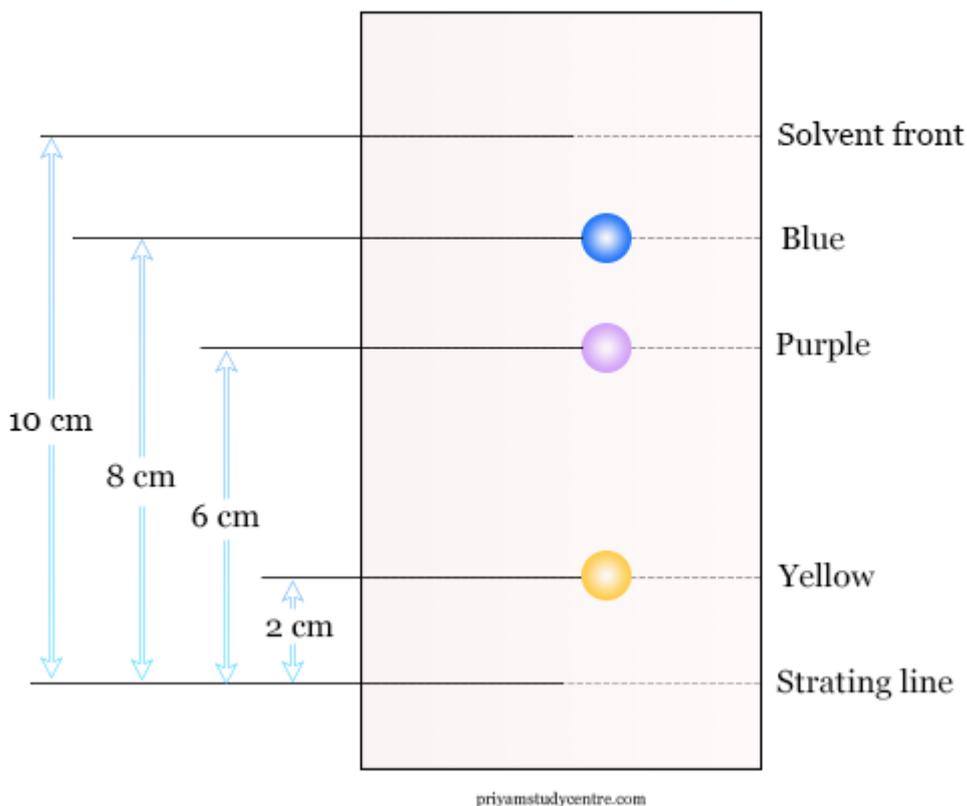
Material required in paper chromatography

- Whatman filter paper strip.
- A mixture of unknown amino acids is dissolved in 10 ml of [water](#).
- Solution of glycine: 5 mg of glycine in 1 ml of water.
- Ninhydrin spray: 200 mg of ninhydrin is dissolved in 99 ml of n-butanol and 1 ml of acetic acid.
- Solvent: n-butanol, acetic acid, and water = 80ml, 20 ml, and 100ml.

Paper chromatography procedure

A line parallel to the short end of the chromatography paper sheet is drawn by a pencil about 10 cm apart. Two points are marked on the Whatman filter paper strip.

The mixture of amino acids is spotted on one mark with no more than 5 mm diameter. Similarly, the second mark is spotted by the standard solution of glycine. The spot is allowed to dry.



The developing solvent is placed in a clean dry glass chamber of a paper chromatographic instrument. The glass chamber is covered with a glass plate having a hole in the middle.

The paper strip hangs from a wire hook dipped into the solution where the spots are just above the solvent front. The solvent rises owing to capillary action on the paper strip. When it almost reaches the point of suspension from the wire hook, the paper strip is carefully taken out from the chamber.

Paper chromatography experiment result

The position of the solvent front on the paper strip is marked. The paper strip is allowed to dry and the [ninhydrin](#) reagent is spread lightly but uniformly. The strip is then heated in an oven at 105 °C for five minutes and the position of the spots is marked.

1. The yellow spot is identified as proline.
2. The spot which moves parallel to the spot of glycine is identified as glycine.
3. The other blush purple spot is phenyl aniline.

Comments:

What is DNA Fingerprinting?

DNA Fingerprinting is a technique that is used to determine the nucleotide sequences of repetitive areas of DNA which are unique to each individual. It is also called DNA profiling or DNA typing.

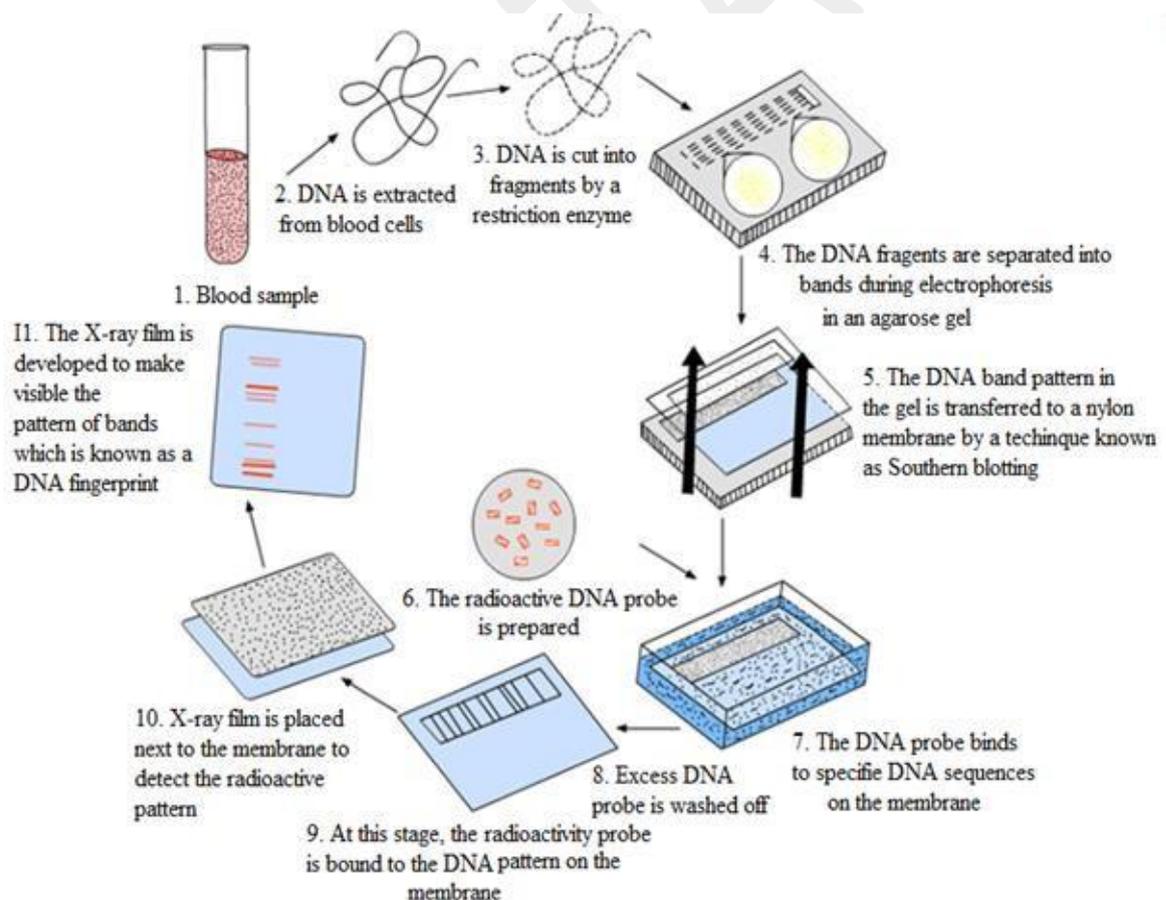
Who Developed DNA Fingerprinting?

The science of DNA Fingerprinting was first used by Sir William Herschel as a method of identification in 1858. Dr. Alec Jeffreys in 1984 invented the DNA fingerprinting technique at the University of Leicester in the United Kingdom to identify the DNA sequences found between the genes to identify the markers for inherited disease so that they can be treated early. At that moment he had no idea the same technique would help to solve the murder cases or paternity cases later. Later, Dr. V.K. Kashyap and Dr. Lalji Singh started the DNA fingerprinting technology in India at CCMB (Centre for Cell and Molecular Biology), Hyderabad.

Principle of DNA Fingerprinting

The principles of DNA fingerprinting are mentioned below:

1. DNA fingerprinting or DNA profiling identifies the combination of DNA sequences which tend to vary greatly between individuals.
2. The most important requirement for DNA fingerprinting is short nucleotide repeats that vary in number from person to person but are inherited. These are called variable number tandem repeats or VNTRs.
3. The DNA fingerprinting technique is based on the theory that except for identical twins (monozygotic twins), no two people possess identical DNA sequences.



PCR or Polymerase Chain Reaction is a technique used in molecular biology to create several copies of a certain DNA segment. This technique was developed in 1983 by Kary Mullis, an American biochemist. PCR has made it possible to generate millions of copies of a small segment of DNA. This tool is commonly used in the molecular biology and biotechnology labs.

Principle of PCR

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA Polymerase synthesises new strands of DNA complementary to the template DNA. The DNA polymerase can add a nucleotide to the pre-existing 3'-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3' prime end of the DNA polymerase.

Components Of PCR

Components Of PCR constitutes the following:

1. **DNA Template**– The DNA of interest from the sample.
2. **DNA Polymerase**– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.
3. **Oligonucleotide Primers**- These are the short stretches of single-stranded DNA complementary to the 3' ends of sense and anti-sense strands.
4. **Deoxyribonucleotide triphosphate**– These provide energy for polymerization and are the building blocks for the synthesis of DNA. These are single units of bases.
5. **Buffer System**– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability.

PCR Steps

The PCR involves three major cyclic reactions:

Denaturation

Denaturation occurs when the reaction mixture is heated to 94°C for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA.

The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

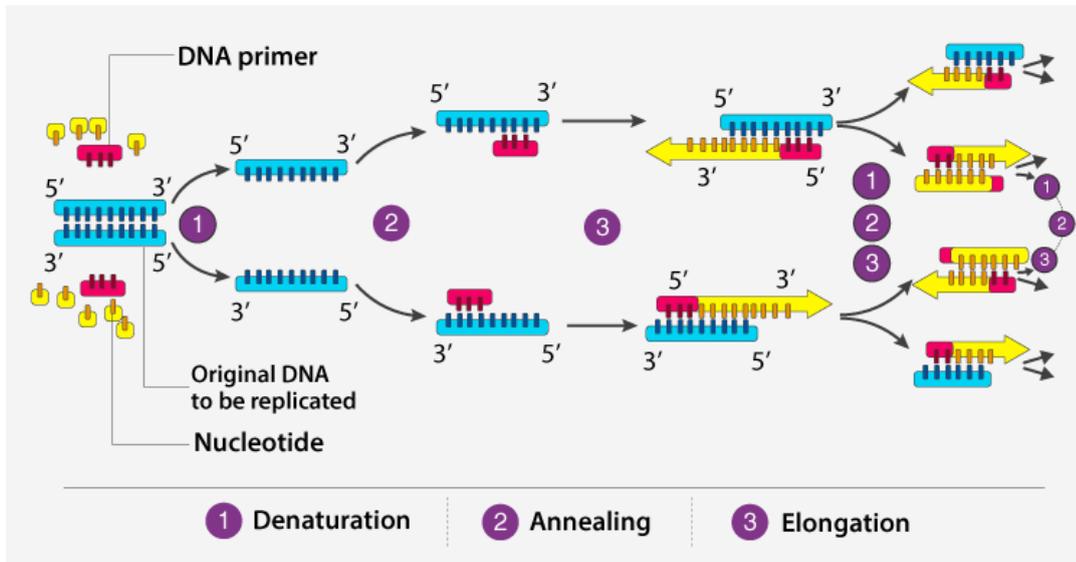
Annealing

The reaction temperature is lowered to 54-60°C for around 20-40 seconds. Here, the primers bind to their complementary sequences on the template DNA.

Primers are single-strand sequences of DNA or RNA around 20 to 30 bases in length.

They serve as the starting point for the synthesis of DNA.

The two separated strands run in the opposite direction and consequently there are two primers- a forward primer and a reverse primer.



Elongation

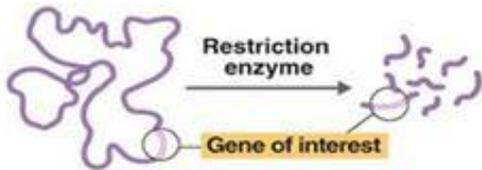
At this step, the temperature is raised to 72-80°C. The bases are added to the 3' end of the primer by the Taq polymerase enzyme.

This elongates the DNA in the 5' to 3' direction. The DNA polymerase adds about 1000bp/minute under optimum conditions.

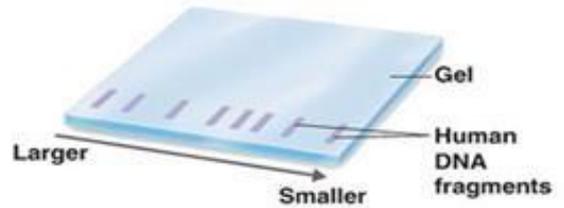
Taq Polymerase can tolerate very high temperatures. It attaches to the primer and adds DNA bases to the single strand. As a result, a double-stranded DNA molecule is obtained.

These three steps are repeated 20-40 times in order to obtain a number of sequences of DNA of interest in a very short time period.

Southern Blotting techniques



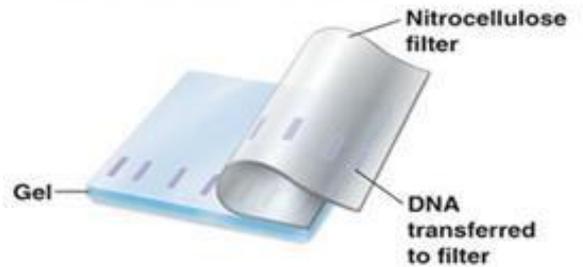
1 DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes.



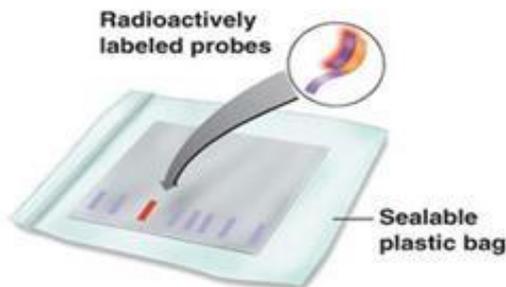
2 The fragments are separated according to size by gel electrophoresis. Each band consists of many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.



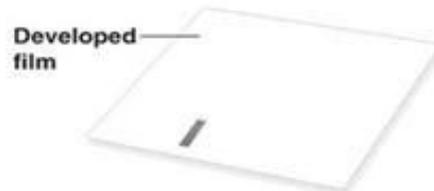
3 The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.



4 This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.

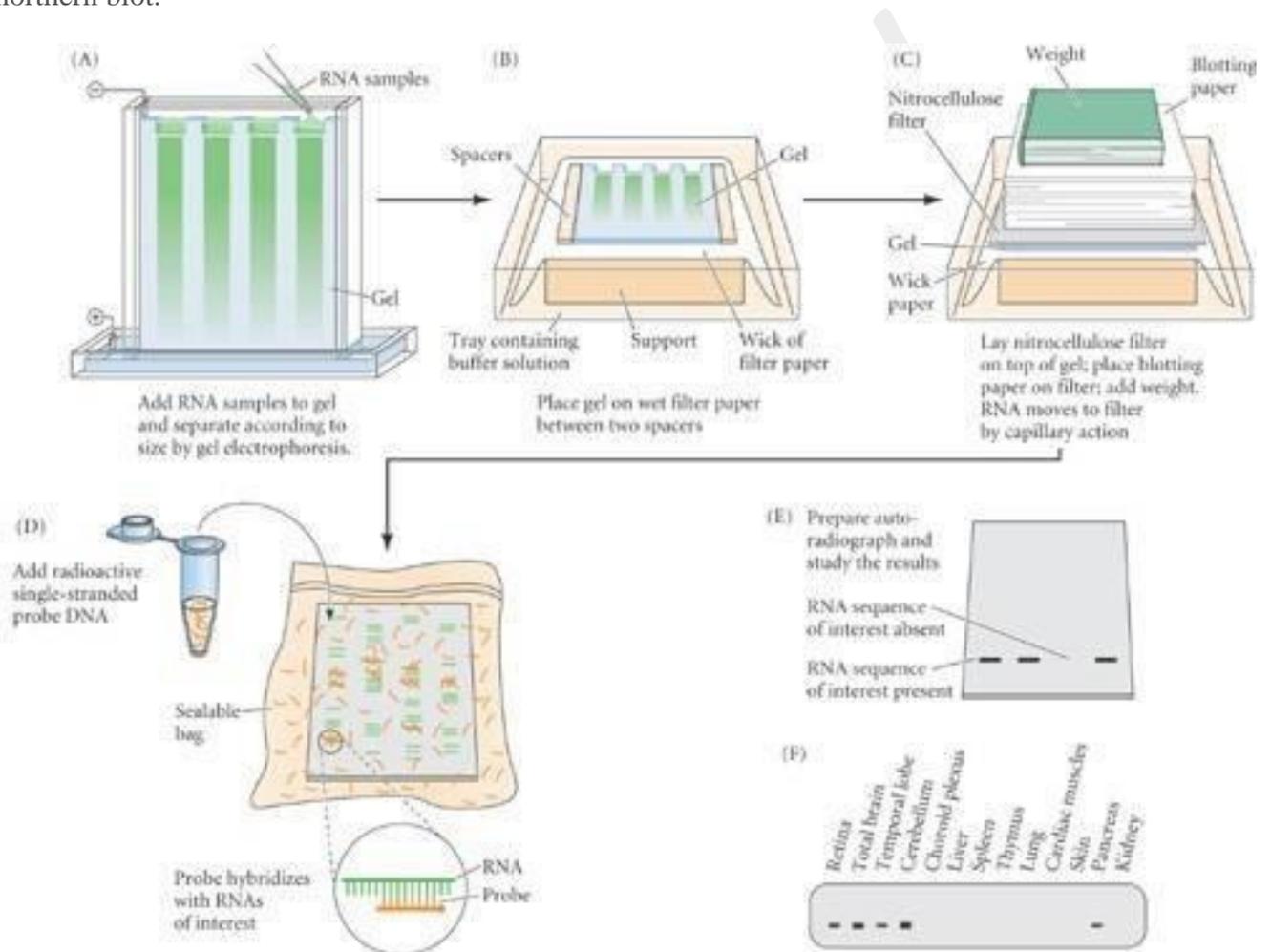


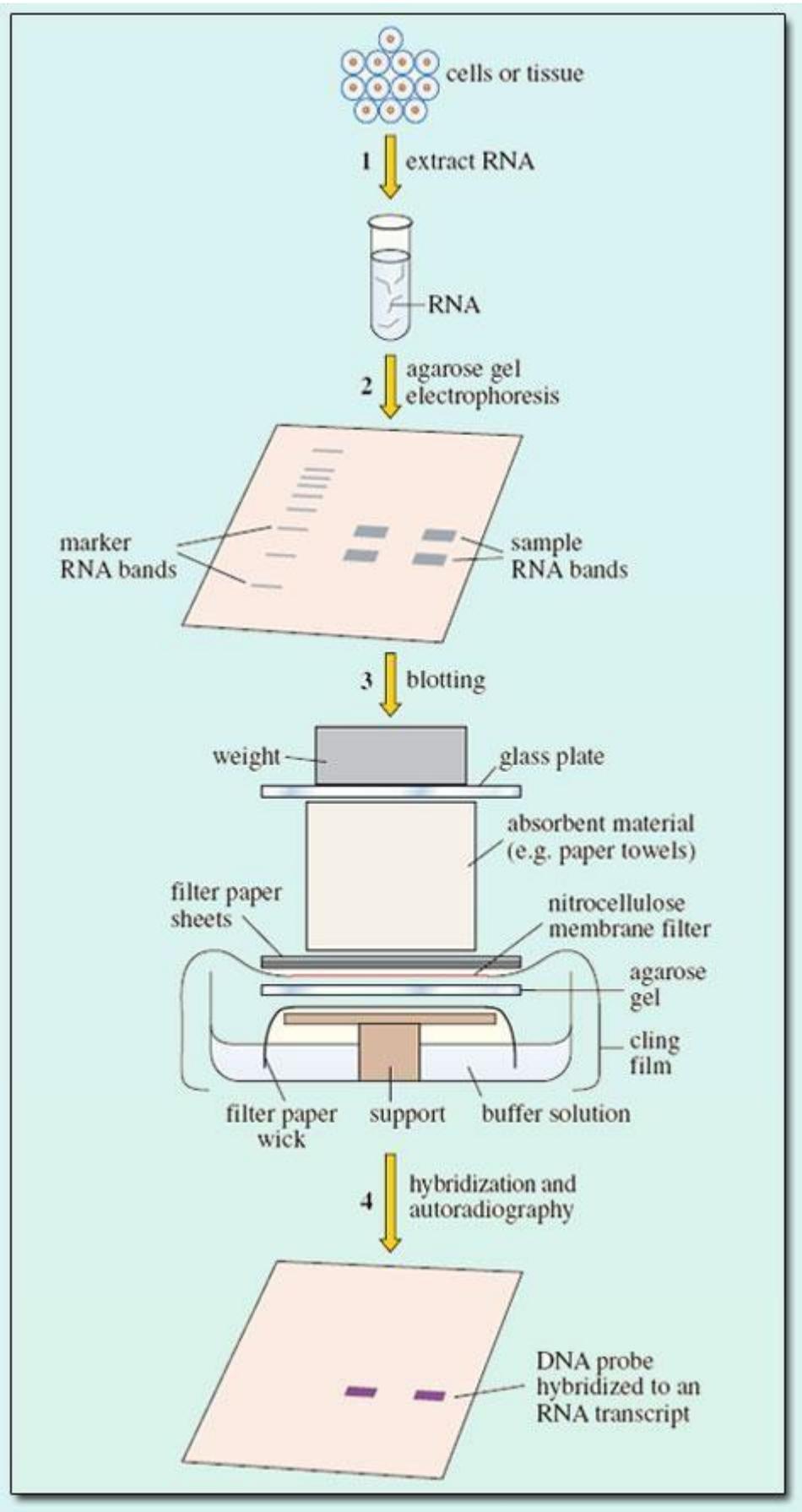
5 The filter is exposed to a radioactively labeled probe for a specific gene. The probe will base-pair (hybridize) with a short sequence present on the gene.



6 The filter is then exposed to X-ray film. The fragment containing the gene of interest is identified by a band on the developed film.

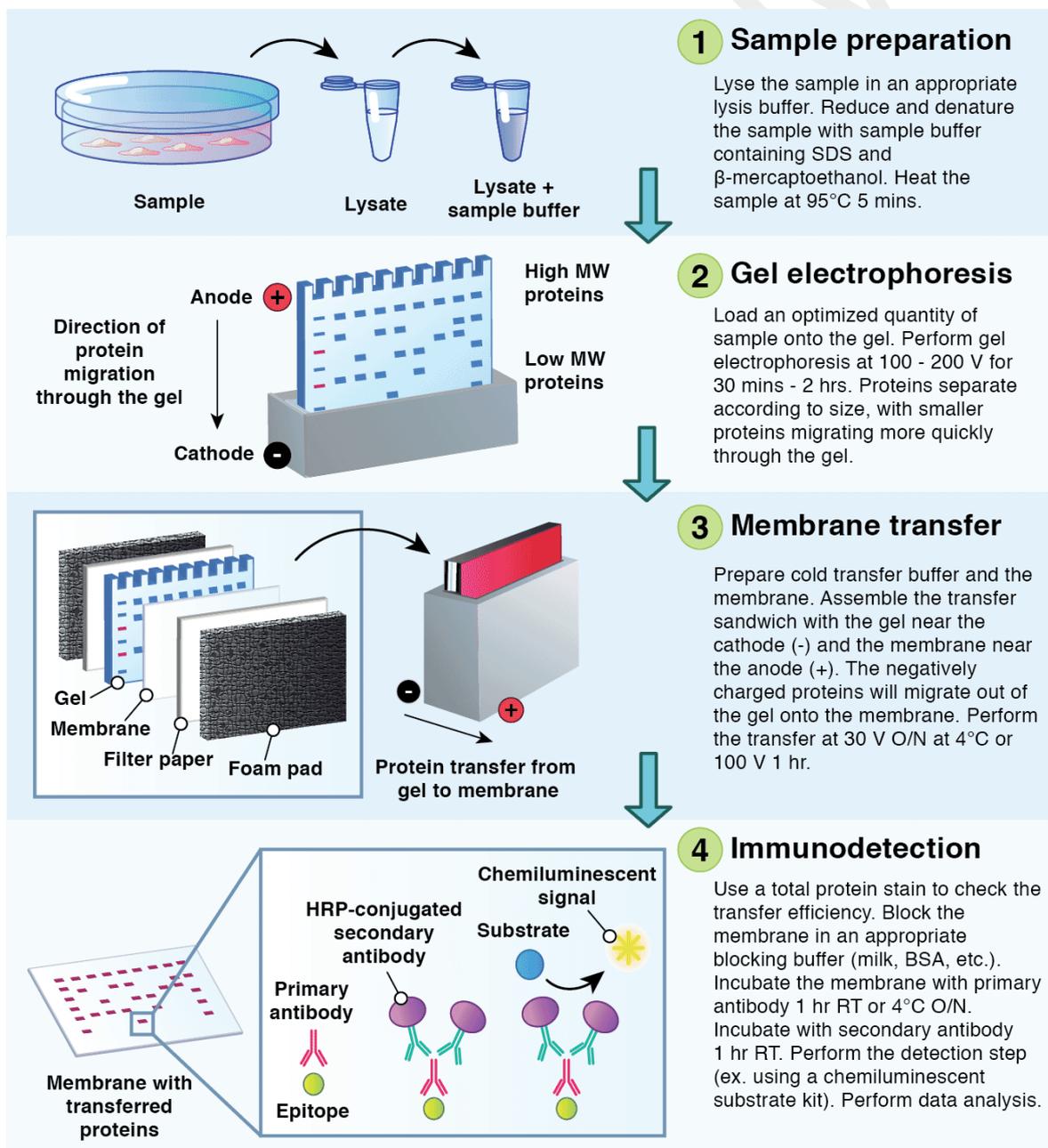
The northern blot technique is used to study gene expression by detection of RNA (or isolated mRNA) in a sample. With northern blotting it is possible to observe cellular control over structure and function by determining the particular gene expression levels during differentiation, morphogenesis, as well as abnormal or diseased conditions. This technique was developed in 1977 by James Alwine, David Kemp and George Stark at Stanford University. Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot. The major difference is that RNA, rather than DNA, is analyzed in the northern blot.





Western blotting. The technique consists of three major processes: 1. Separation of proteins by size (Electrophoresis). 2. Transfer to a solid support (Blotting) 3. Marking target protein using a proper primary and secondary antibody to visualize (Detection).

It utilizes SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis), a type of gel electrophoresis to first separate various proteins in a mixture on the basis of their shape and size. The protein bands thus obtained are transferred onto a nitrocellulose or nylon membrane where they are “probed” with antibodies specific to the protein to be detected. The antigen–antibody complexes that form on the band containing the protein recognized by the antibody can be visualized in a variety of ways. If the protein of interest is bound by a radioactive antibody, its position on the blot can be determined by exposing the membrane to a sheet of X-ray film, a procedure called autoradiography.



SDS-PAGE Set Up:

