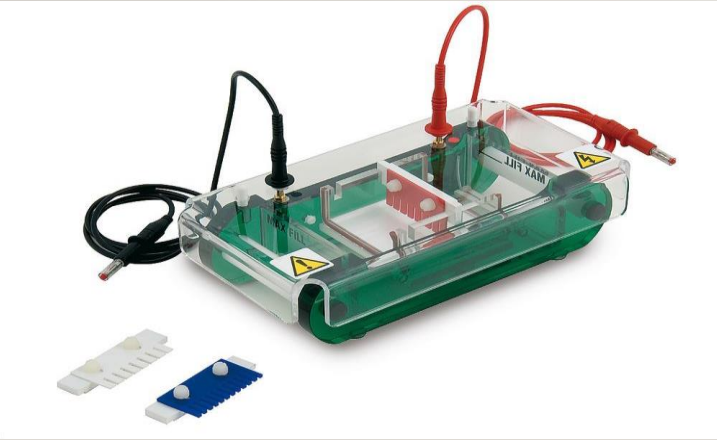
ELECTROPHORESIS



BIOCHEMISTRY DEPARTMENT

FACULTY OF PHARMACY

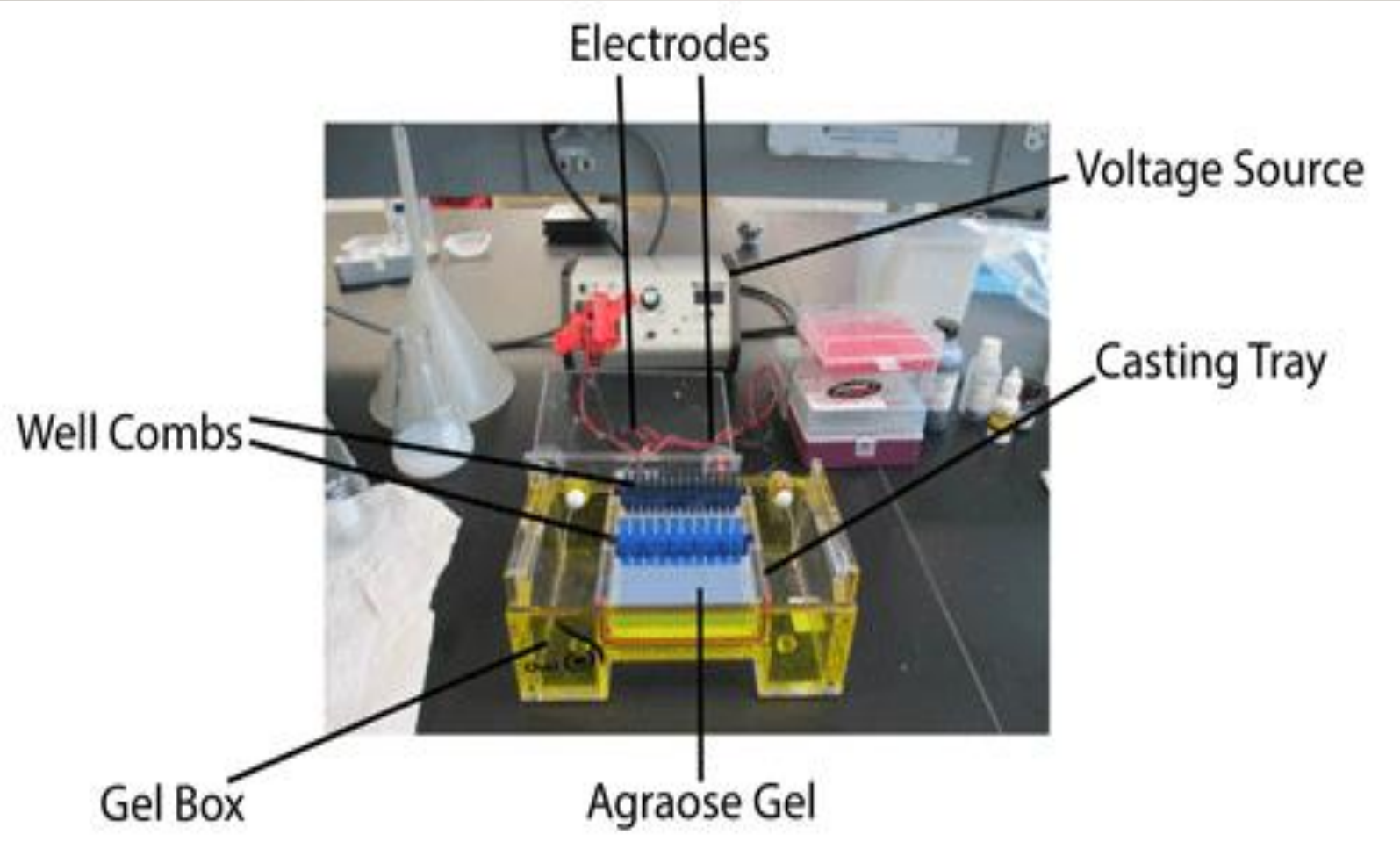
SOUTH VALLEY UNIVERSITY

PHARM D

SECTION 7

Introduction

- Electrophoresis is the separation of charged molecules in an applied electric field.
- The relative mobility of individual molecules depends on several factors. The most important of which are net charge, charge/mass ratio, molecular shape and the temperature, porosity and viscosity of the matrix through which the molecule migrates.
- Complex mixtures can be separated to very high resolution by this process.


Electrodes
Voltage Source
Casting Tray
Well Combs
Gel Box
Agraose Gel

Gel electrophoresis

- Hydrated gel networks have many desirable properties for electrophoresis. They allow a wide variety of mechanically stable experimental formats such as horizontal/vertical electrophoresis in slab gels or electrophoresis in tubes or capillaries.
- The mechanical stability also facilitates post electrophoretic manipulation making further experimentation possible such as blotting.

Gel types

- The most commonly used materials for the separation of nucleic acids and proteins are agarose and acrylamide.

a) Agarose: The most widely used polysaccharide gel matrix nowadays is that formed with agarose. This is a polymer composed of a repeating disaccharide unit called agarobiose. This gel has found wide spread use especially in the separation of DNA molecules (although it may also be used in some electrophoretic procedures involving protein samples).

N.B. Because of the uniform charge distribution in nucleic acids, it is possible accurately to determine DNA molecular masses based on mobility in agarose gels.

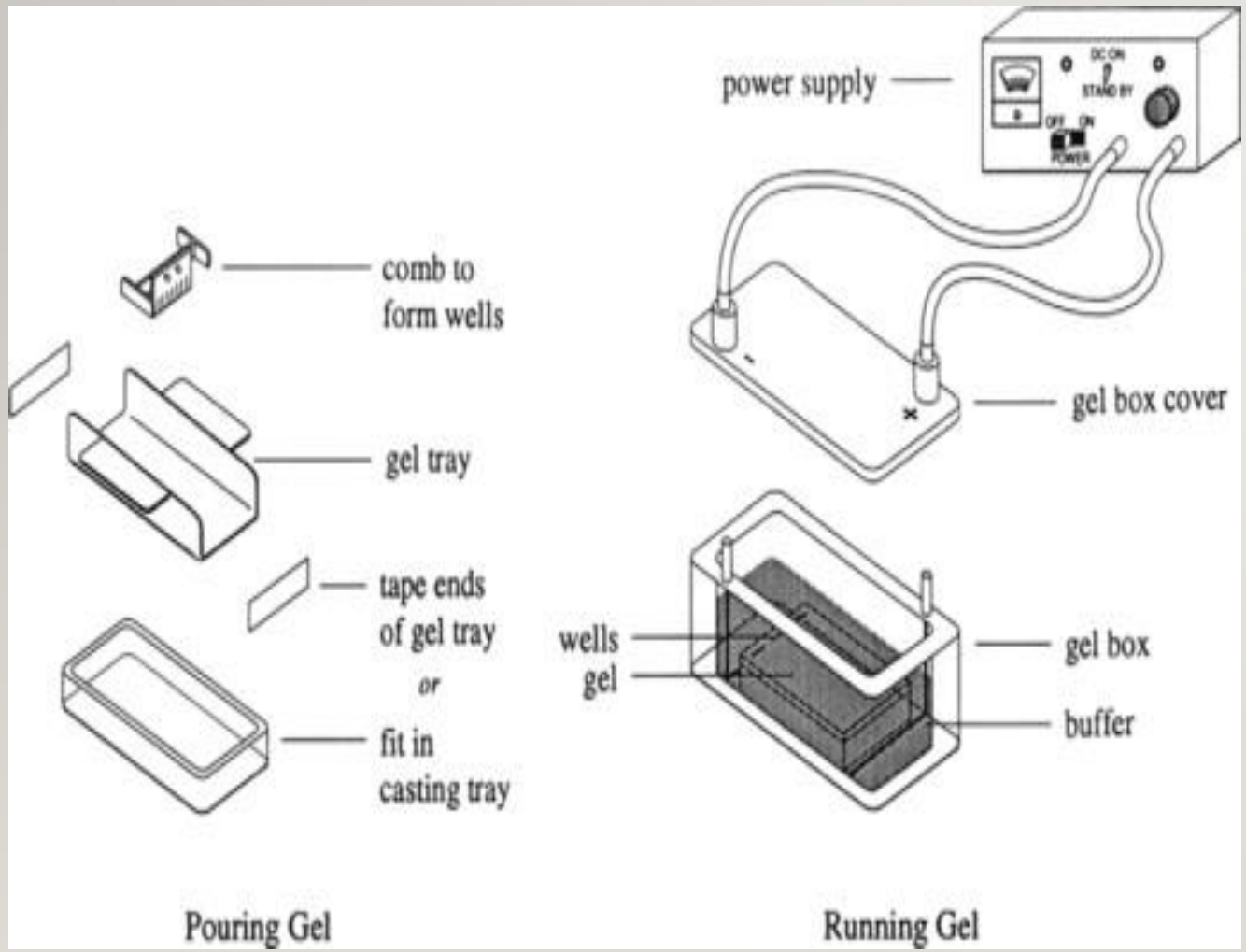
Gel types

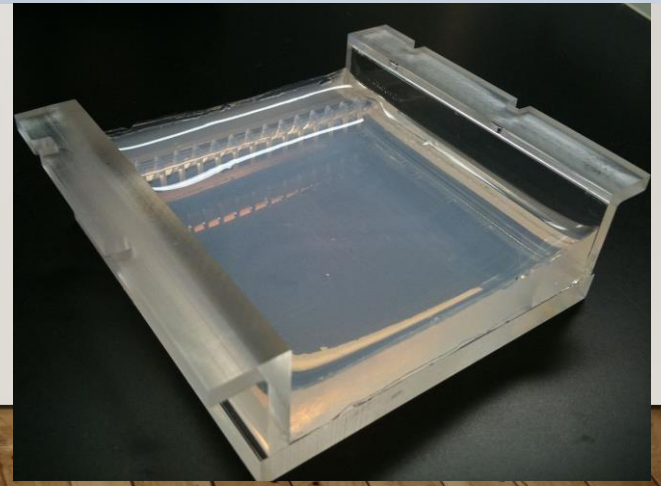
b) Acrylamide: A stronger gel suitable for electrophoretic separation of both proteins and nucleic acids may be formed by the polymerization of acrylamide. The inclusion of a small amount of acrylamide cross linked by a methylene bridge (N,N′ methylene bisacrylamide) allows formation of a cross linked gel with a highly-controlled porosity which is also mechanically strong and chemically inert. For separation of proteins, the ratio of acrylamide is usually 40:1 while for DNA separation it is 19:1. Such gels are suitable for high-resolution separation of DNA and proteins.

Staining of gel

One of the most important aspects of gel electrophoresis technique is staining. Once sample molecules have separated in the gel matrix it is necessary to visualize their position. This is achieved by staining with an agent appropriate for the sample.

The equipment and supplies of standard agarose gels





The equipment and supplies of standard agarose gels

1. An electrophoresis chamber and power supply.
2. Gel casting trays, which are available in a variety of sizes and composed of UV-transparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
3. Sample combs, around which molten medium is poured to form sample wells in the gel.
4. Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).

The equipment and supplies of standard agarose gels

5. Loading buffer, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.

Preparation and running of standard agarose gels

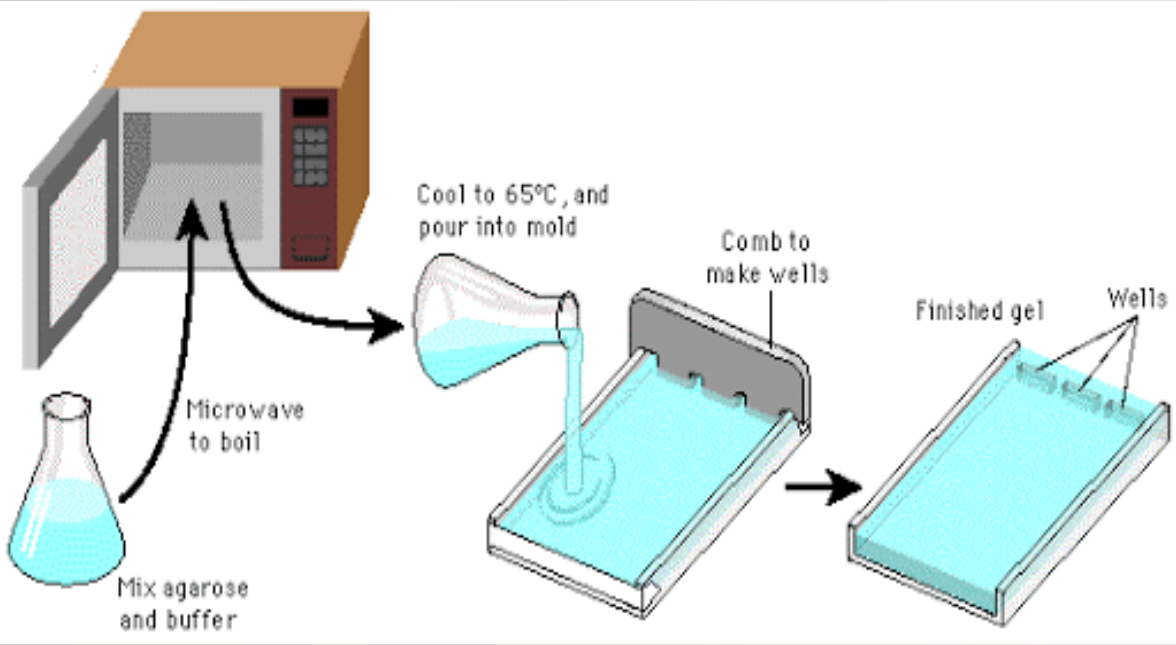
6. Staining: DNA molecules are easily visualized under an ultraviolet lamp when electrphoresed in the presence of the extrinsic fluoro-ethidium bromide.

NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling.

7. Transilluminator (an ultraviolet light box), which is used to visualize stained DNA in gels.

NOTE: always wear protective eyewear when observing DNA on a Transilluminator to prevent damage to the eyes from UV light.

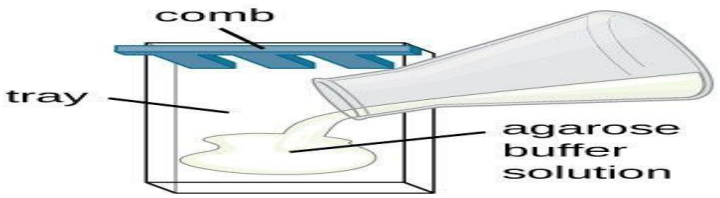
STEPS


Cool to 65°C, and
pour into mold
Comb to
make wells
Finished gel
Wells
Microwave
to boil
Mix agarose
and buffer

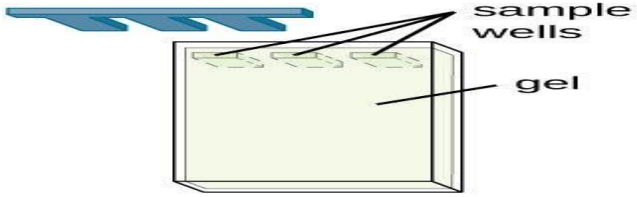

EtBr
Add 2.5µL Ethidium Bromide and Stir

STEPS

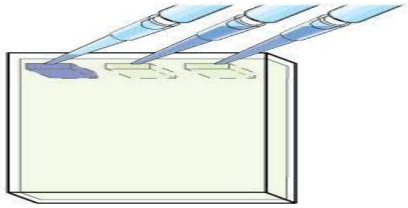
1. An agarose and buffer solution is poured into a plastic tray. A comb is placed into the tray on one end.



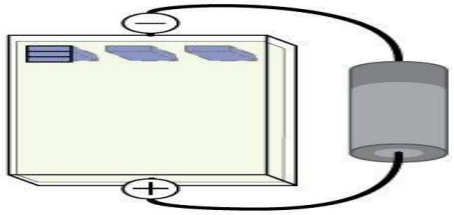
2. The agarose polymerizes into a gel as it cools. The comb is removed from the gel to form wells for samples.



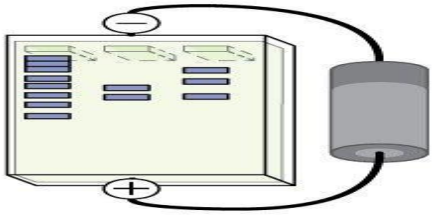
3. DNA samples colored with a tracking dye are pipetted into the wells.



4. The tray is placed into a chamber that generates electric current through the gel. The negative electrode is placed on the side nearest the samples. The positive electrode is placed on the other side.

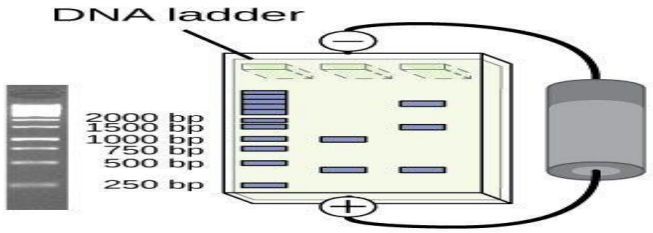


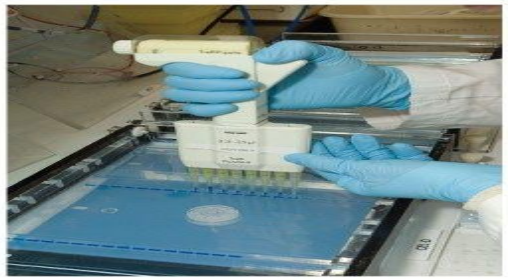
5. DNA has a negative charge and will be drawn to the positive electrode. Smaller DNA molecules will be able to travel faster through the gel.



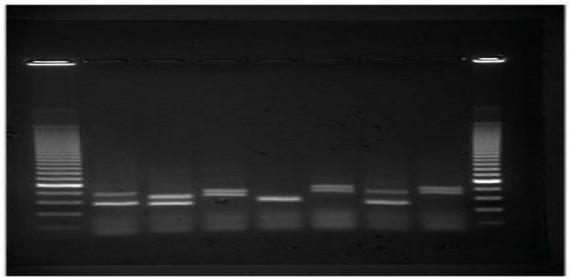
(a)

6. One well, called a DNA ladder, will contain DNA fragments of known sizes. This ladder is used to determine the sizes of other samples.





(b)



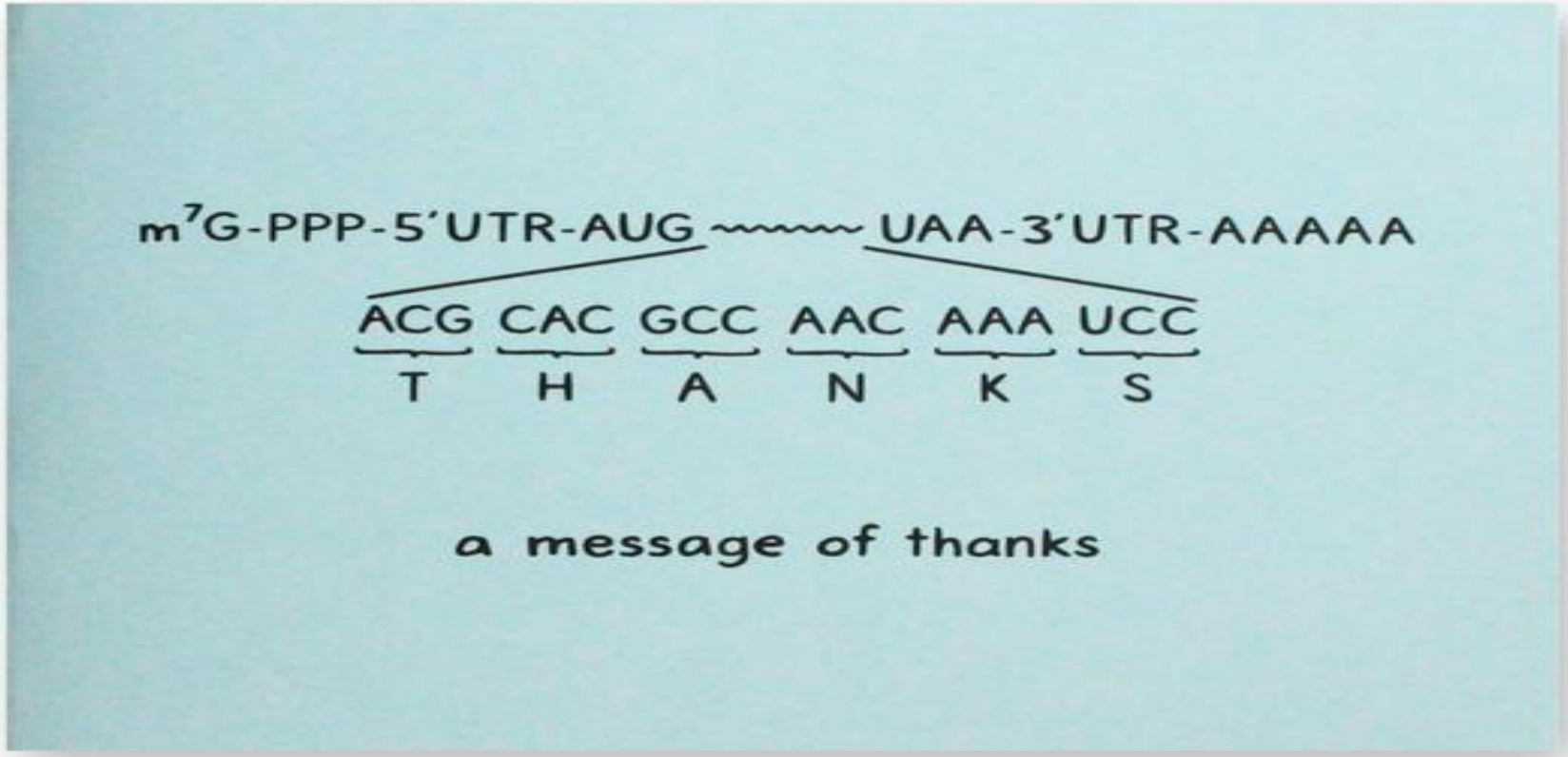
(c)

STEPS

1. To prepare gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and heated in a microwave oven to melt it.
2. Ethidium bromide is added to the gel (final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis.
3. After cooling the solution to about 60oC, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.
4. After the gel has solidified, the comb is removed, taking care not to rip the bottom of the wells. The gel, still in plastic tray, is inserted horizontally into the electrophoresis chamber and is covered with buffer.

STEPS

5. Samples containing DNA mixed with loading buffer are then pipetted into the sample wells, the lid and power leads are placed on the apparatus , and a current is applied. The current flow can be confirmed by observing bubbles coming off the electrodes.
6. DNA will migrate towards the positive electrode, which is usually colored red, in view of its negative charge.
7. The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes like bromophenol blue and xylene cyanol dyes.


m7G-PPP-5'UTR-AUG ~~~~ UAA-3'UTR-AAAAA
ACG CAC GCC AAC AAA UCC
T H A N K S
a message of thanks