# Gel Electrophoresis

By-

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# Introduction

- The initial impetus for gene manipulation in vitro came about in the early 1970s with the simultaneous development of techniques for:
  - genetic transformation of Escherichia coli;
  - cutting and joining DNA molecules;
  - monitoring the cutting and joining reactions.
- The cutting and joining reactions must be readily monitored.
- The progress of the first experiments on cutting and joining of DNA molecules was monitored by velocity sedimentation in sucrose gradients.
- However, this has been entirely superseded by gel electrophoresis.

# Types of Gel Electrophoresis

- Horizontal
- Vertical (SDS-PAGE)

# Gel electrophoresis (Horizontal)

- Gel electrophoresis is used to separate different nucleic acid molecules on the basis of their size.
- Gel electrophoresis is not only used as an analytical method, it is also routinely used preparatively for the purification of specific DNA fragments.
- The gel is composed of polyacrylamide or agarose.
- Agarose is convenient for separating DNA fragments ranging in size from a few hundred base pairs to about 20 kb.
- Polyacrylamide is preferred for smaller DNA fragments.

# Agarose gel electrophoresis

- Agarose is isolated from the seaweed genera *Gelidium* and *Gracilaria*, and consists of repeated agarobiose (L- and D-galactose) subunits.
- Agarose is a linear polysaccharide made up from alternating D-galactose and 3,6-anhydro-alpha-L-galactopyranose residues joined by alpha-(1->3)- and beta-(1->4)-linkages.
- The agarose gel has a gelling temperature of 35–42 °C and a melting temperature of 85–95 °C.
- Low-melting and low-gelling agaroses made through chemical modifications are also available.
- During gelation, agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties.

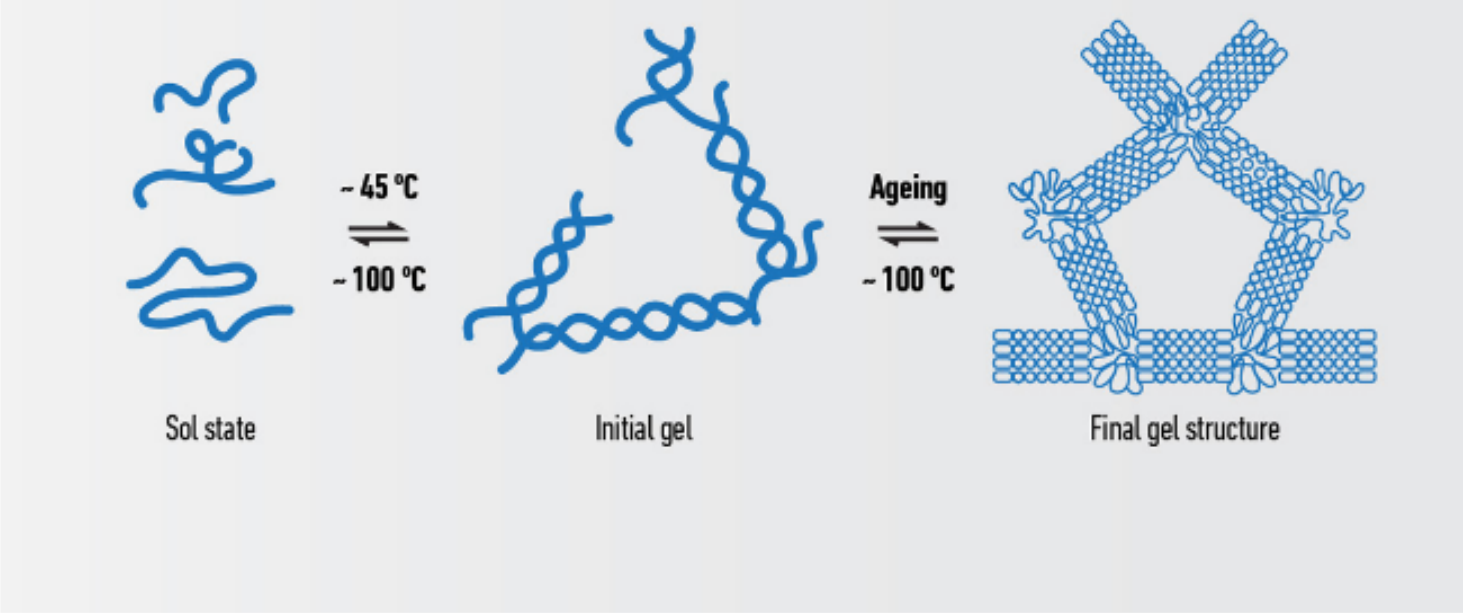



**Figure 1:** Pore formation and temperature-induced state transition in agarose gel.

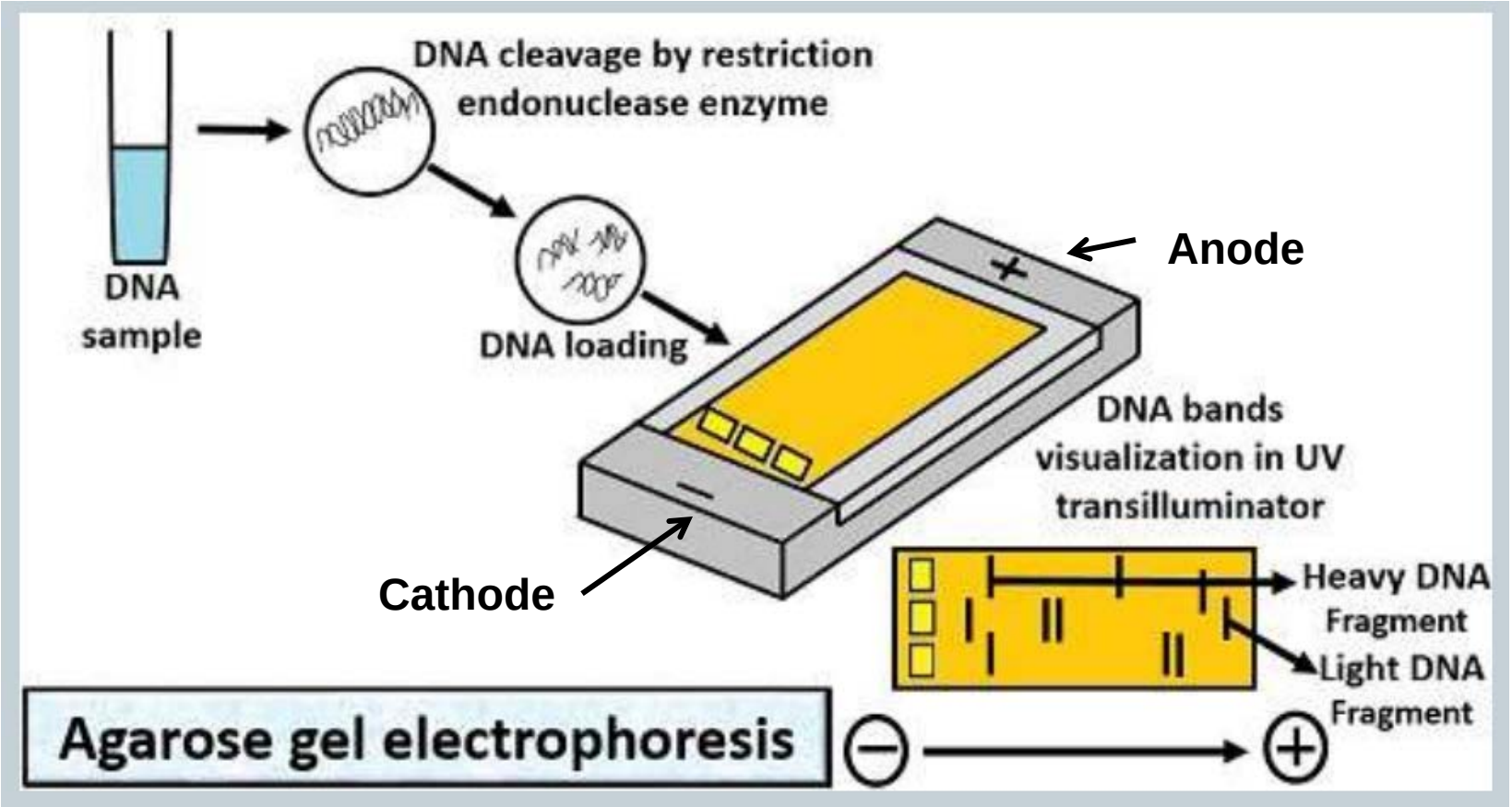
- To separate DNA using agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current applied.
- The phosphate backbone of the DNA (and RNA) molecule is negatively charged, therefore when placed in an electric field, DNA fragments will migrate to the positively charged anode.
- Because DNA has a uniform mass/charge ratio, DNA molecules are separated by size within an agarose gel in a pattern such that the distance traveled is inversely proportional to the log of its molecular weight.

# Agarose

- The agarose polymer contains charged groups, in articular pyruvate and sulphate.
- These negatively charged groups create a flow of water in the opposite direction to the movement of DNA in a process called **electroendosmosis**(EEO), and can therefore retard the movement of DNA and cause blurring of bands.
- Higher concentration gels would have higher electroendosmotic flow.
- Agarose can be modified to create low melting agarose through hydroxyethylation.
- The lower sulphate content of low EEO agarose, particularly low-melting point (LMP) agarose, is also beneficial in cases where the DNA extracted from gel is to be used for further manipulation as the presence of contaminating sulphates may affect some subsequent procedures, such as ligation and PCR.
- Zero EEO agaroses however are undesirable for some applications as they may be made by adding positively charged groups and such groups can affect subsequent enzyme reactions.
- Electroendosmosis is a reason agarose is used in preference to agar as the agaropectin component in agar contains a significant amount of negatively charged sulphate and carboxyl groups.
- The removal of agaropectin in agarose substantially reduces the EEO, as well as reducing the non-specific adsorption of biomolecules to the gel matrix.

# Models for DNA movement through an agarose gel

- A widely accepted one is the **Ogston model** which treats the polymer matrix as a sieve.
- A globular protein or a random coil DNA moves through the interconnected pores, and the movement of larger molecules is more likely to be impeded and slowed down by collisions with the gel matrix, and the molecules of different sizes can therefore be separated in this sieving process.
- The Ogston model however breaks down for large molecules whereby the pores are significantly smaller than size of the molecule.
- For DNA molecules of size greater than 1 kb, a **reptation model** is most commonly used.
- This model assumes that the DNA can crawl in a "snake-like" fashion (hence "reptation") through the pores as an elongated molecule.
- A **biased reptation model** applies at higher electric field strength, whereby the leading end of the molecule become strongly biased in the forward direction and pulls the rest of the molecule along.
- Real-time fluorescence microscopy of stained molecules, however, showed more subtle dynamics during electrophoresis, with the DNA showing considerable elasticity as it alternately stretching in the direction of the applied field and then contracting into a ball, or becoming hooked into a U-shape when it gets caught on the polymer fibres.




- The rate of migration of a DNA molecule through a gel is determined by the following:

  1) size of DNA molecule;
  2) agarose concentration;
  3) DNA conformation;
  4) voltage applied,
  5) presence of ethidium bromide,
  6) type of agarose, and
  7) electrophoresis buffer.

# Protocol

1. **Preparation of the Gel**

- Agarose gels are prepared using a w/v percentage solution. The concentration of agarose in a gel will depend on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%.
- Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).
- Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.
- Add ethidium bromide (EtBr) to a concentration of 0.5 µg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 µg/ml EtBr for 15-30 min, followed by destaining in running buffer for an equal length of time.
- Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells.
- Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use.

# Ethidium bromide

- EtBr works by intercalating itself in the DNA molecule in a concentration-dependent manner.
- When exposed to a short wave ultraviolet light source (transilluminator), electrons in the aromatic ring of the ethidium molecule are activated, which leads to the release of energy (light) as the electrons return to the ground state.
- This allows for an estimation of the amount of DNA in any particular DNA band based on its intensity.
- **Ethidium bromide is a suspect mutagen and carcinogen so must be handled cautiously.*
- Alternative stains for DNA in agarose gels include SYBR Gold, SYBR green, crystal violet, and methyl blue.
- The sensitivities of methylene blue and crystal violet are low compared with ethidium bromide.
- SYBR gold and SYBR green are highly sensitive but more expensive than EtBr.

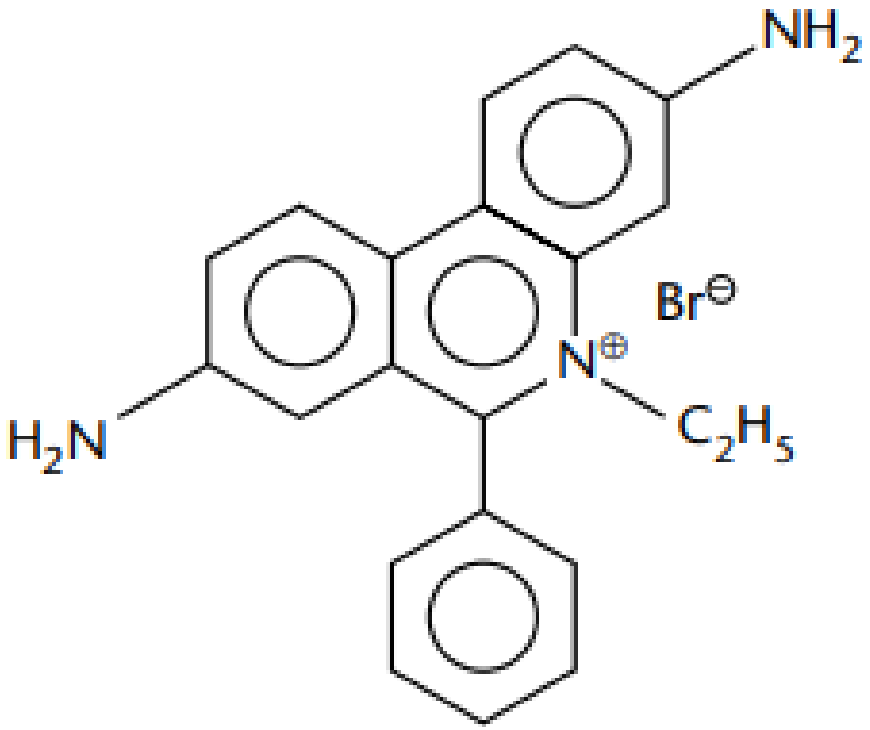



**Fig. 2.3** Ethidium bromide.

# Protocol

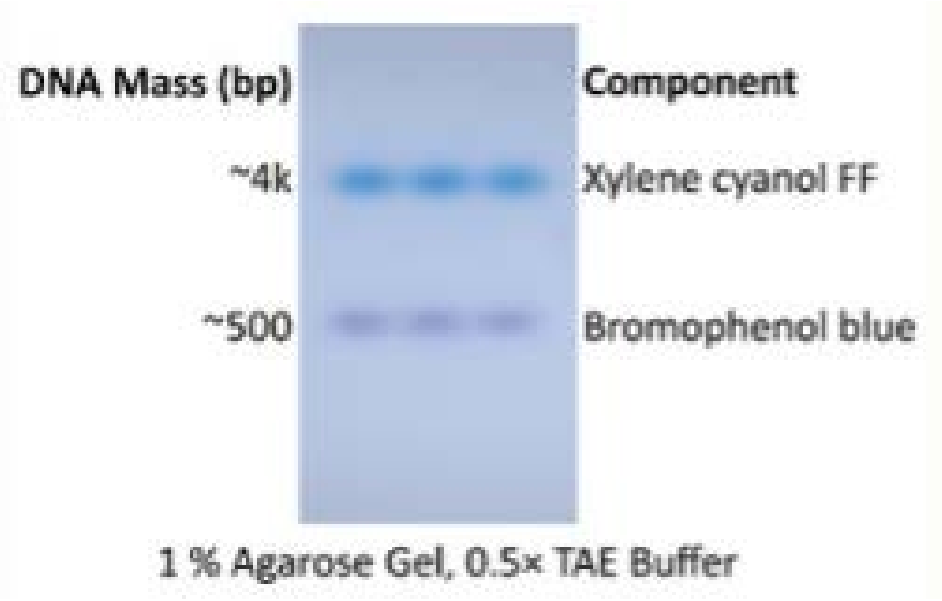
**2. Setting up of Gel Apparatus and Separation of DNA Fragments**

- Add loading dye to the DNA samples to be separated. Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol). Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel. Bromophenol Blue, and Xylene Cyanol which migrate similar to DNA bands of 500bp, and 4kb respectively in a 1% agarose gel.
- Program the power supply to desired voltage (1-5V/cm between electrodes).
- Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.
- Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working.
- Remove the lid. Slowly and carefully load the DNA sample(s) into the gel.
- Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads).
- Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

# Protocol

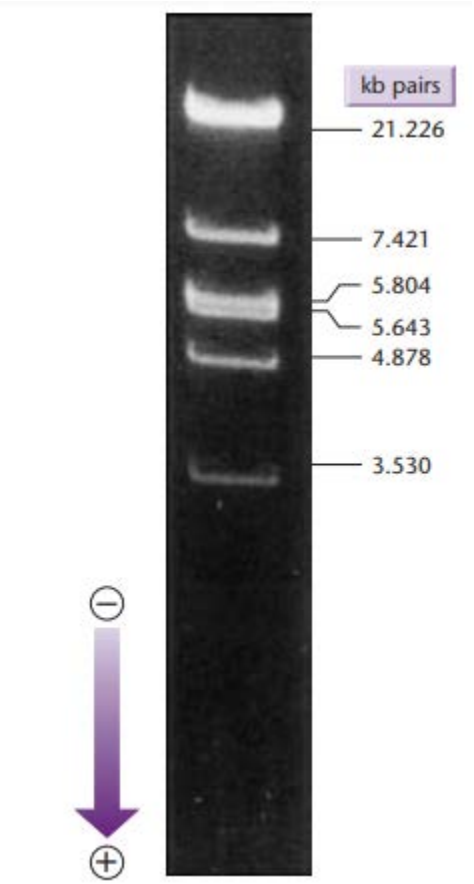
**3. Observing Separated DNA fragments**

- When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.
- Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.
- Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system. DNA bands should show up as orange fluorescent bands. Take a picture of the gel.
- Properly dispose of the gel and running buffer per institution regulations.



# Figure 1

- Electrophoresis of DNA in agarose gels.
- The direction of migration is indicated by the arrow.
- DNA bands have been visualized by soaking the gel in a solution of ethidium bromide (see Fig. 1), which complexes with DNA by intercalating between stacked base pairs, and photographing the orange fluorescence which results upon ultraviolet irradiation.



# Points of consideration

- An agarose gel is a complex network of polymeric molecules whose average pore size depends on the buffer composition and the type and concentration of agarose used.
- In general, the higher the concentration of agarose, the smaller the pore size.
- Traditional agarose gels are most effective at the separation of DNA fragments between 100 bp and ~20 kb.
- The larger the pore size of the gel, the greater the ball of DNA which can pass through and hence the larger the molecules which can be separated.
- Once the globular volume of the DNA molecule exceeds the pore size, the DNA molecule can only pass through by reptation.
- This occurs with molecules about 20 kb in size and it is difficult to separate molecules larger than this without recourse to pulsed electrical fields.

# Pulsed-field gel electrophoresis (PFGE)

- In pulsed-field gel electrophoresis (PFGE) molecules as large as 10 Mb can be separated in agarose gels.
- To separate DNA fragments larger than 25 kb, one will need to use pulse field gel electrophoresis, which involves the application of alternating current from two different directions.
- In this way larger sized DNA fragments are separated by the speed at which they reorient themselves with the changes in current direction.
- DNA to periodically alter its direction of migration by regular changes in the orientation of the electric field with respect to the gel.
- With each change in the electric-field orientation, the DNA must realign its axis prior to migrating in the new direction.
- Electric-field parameters, such as the direction, intensity, and duration of the electric field, are set independently for each of the different fields and are chosen so that the net migration of the DNA is down the gel.
- The difference between the direction of migration induced by each of the electric fields is the reorientation angle and corresponds to the angle that the DNA must turn as it changes its direction of migration each time the fields are switched.

# Contour-clamped homogeneous electrical-field (CHEF) electrophoresis

- A major disadvantage of PFGE, as originally described, is that the samples do not run in straight lines.
- This makes subsequent analysis difficult.
- This problem has been overcome by the development of improved methods for alternating the electrical field.
- The most popular of these is contour-clamped homogeneous electrical-field (CHEF) electrophoresis.
- In early CHEF-type systems (Fig. 2.2) the reorientation angle was fixed at 120°.
- However, in newer systems, the reorientation angle can be varied and it has been found that for whole-yeast chromosomes the migration rate is much faster with an angle of 106°.
- Fragments of DNA as large as 200–300 kb are routinely handled in genomics work and these can be separated in a matter of hours using CHEF systems with a reorientation angle of 90° or less.

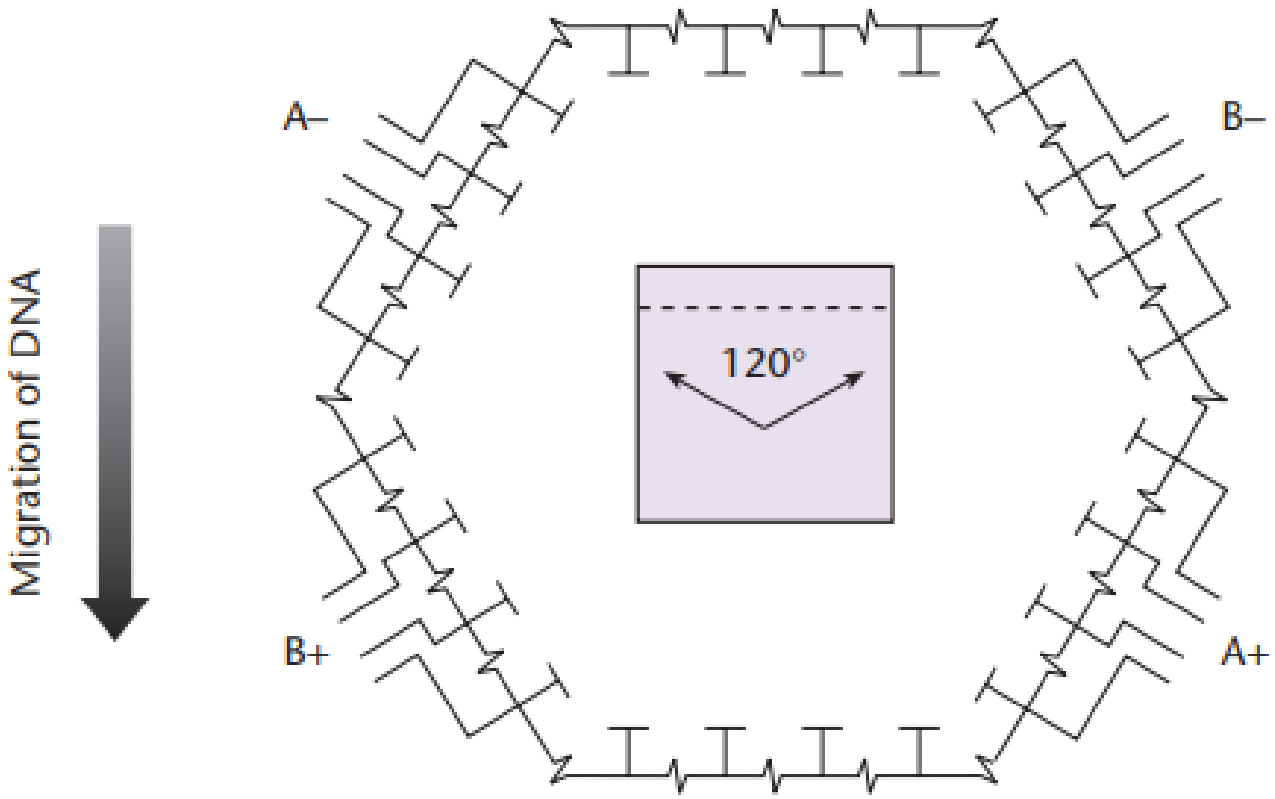



**Fig. 2.2** Schematic representation of CHEF (contour-clamped homogeneous electrical field) pulsed-field gel electrophoresis.

# Polyacrylamide gel matrix

- DNA fragments smaller than 100 bp are more effectively separated using polyacrylamide gel electrophoresis.
- Unlike agarose gels, the polyacrylamide gel matrix is formed through a free radical driven chemical reaction.
- These thinner gels are of higher concentration, are run vertically and have better resolution.

# Molecular Weight Detection

- Aaij and Borst (1972) showed that the migration rates of DNA molecules were inversely proportional to the logarithms of their molecular weights.
- Subsequently, Southern (1979a,b) showed that plotting fragment length or molecular weight against the reciprocal of mobility gives a straight line over a wider range than the semilogarithmic plot.
- In any event, gel electrophoresis is frequently performed with marker DNA fragments of known size, which allows accurate size determination of an unknown DNA molecule by interpolation.
- A particular advantage of gel electrophoresis is that the DNA bands can be readily detected at high sensitivity.
- Traditionally, the bands of DNA have been stained with the intercalating dye ethidium bromide and as little as 0.05 μg of DNA can be detected as visible fluorescence when the gel is illuminated with ultraviolet light.

# Applications of Gel Electrophoresis

- In addition to resolving DNA fragments of different lengths, gel electrophoresis can be used to separate different molecular configurations of a DNA molecule (eg. plasmids).
- Gel electrophoresis can also be used for investigating protein–nucleic acid interactions in the so-called gel retardation or band shift assay.
- It is based on the observation that binding of a protein to DNA fragments usually leads to a reduction in electrophoretic mobility.
- The assay typically involves the addition of protein to linear double-stranded DNA fragments, separation of complex and naked DNA by gel electrophoresis and visualization.

# Plasmids

- Most plasmids exist as double-stranded circular DNA molecules.
- If both strands of DNA are intact circles the molecules are described as covalently closed circles or CCC DNA.
- If only one strand is intact, then the molecules are described as open circles or OC DNA.
- When isolated from cells, covalently closed circles often have a supercoiled configuration.
- Because of their different structural configurations, super coiled and OC DNA separate upon electrophoresis in agarose gels.
- Addition of an intercalating agent, such as ethidium bromide, to supercoiled DNA causes the plasmid to unwind.
- If excess ethidium bromide is added, the plasmid will rewind in the opposite direction (Fig. 4.3).
- Use of this fact is made in the isolation of plasmid DNA.

# Electrophoresis of DNA of different molecular configurations

- (A) Open circular (OC) and supercoiled (SC) forms of a plasmid of 6.4 kb pairs.
- Note that the compact supercoils migrate considerably faster than open circles.
- (B) Linear plasmid (L) DNA is produced by treatment of the preparation shown in lane (A) with EcoRI, for which there is a single target site.
- Under the conditions of electrophoresis employed here, the linear form migrates just ahead of the open circular form.