Electrophoresis

By

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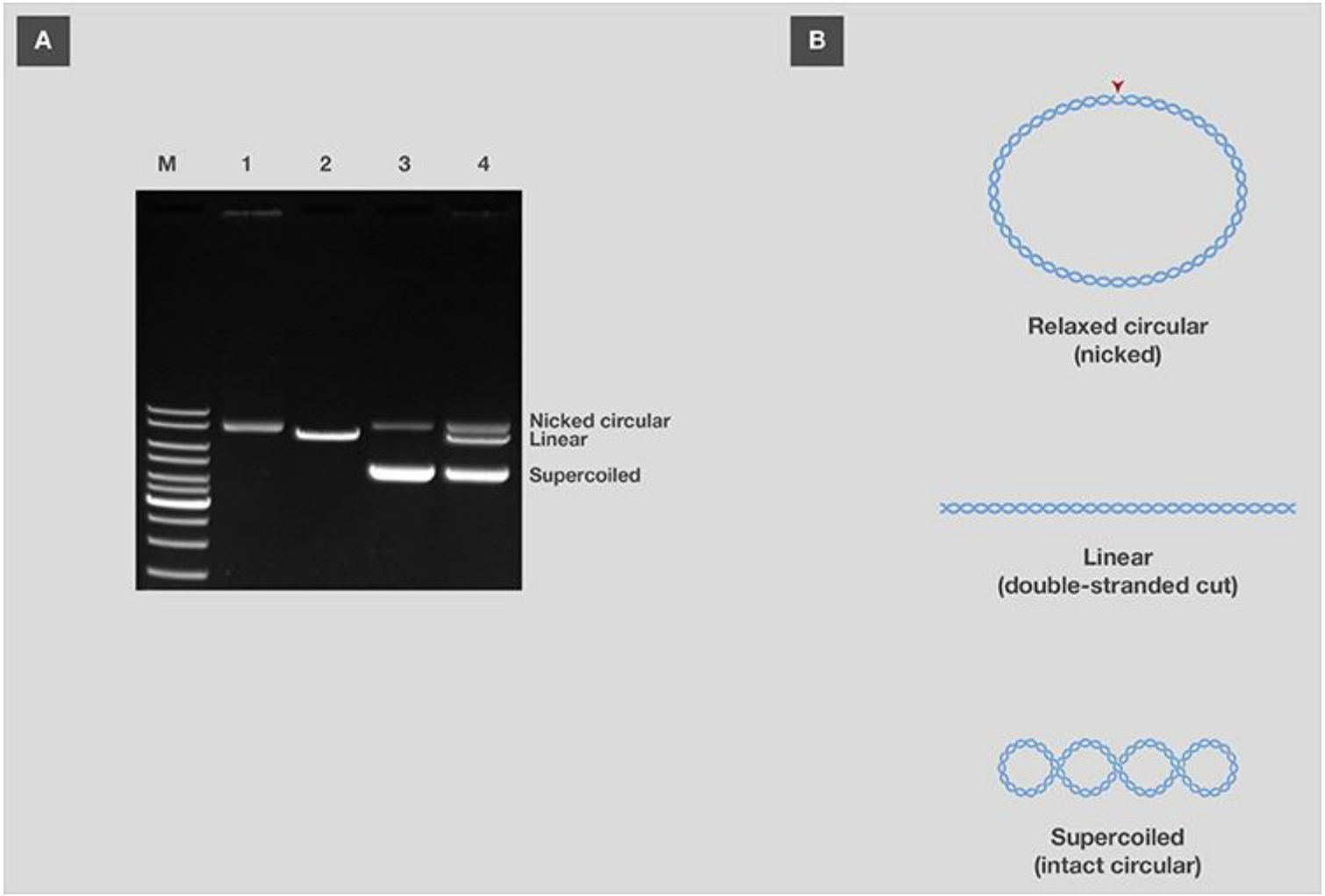
Basic principle of gel electrophoresis

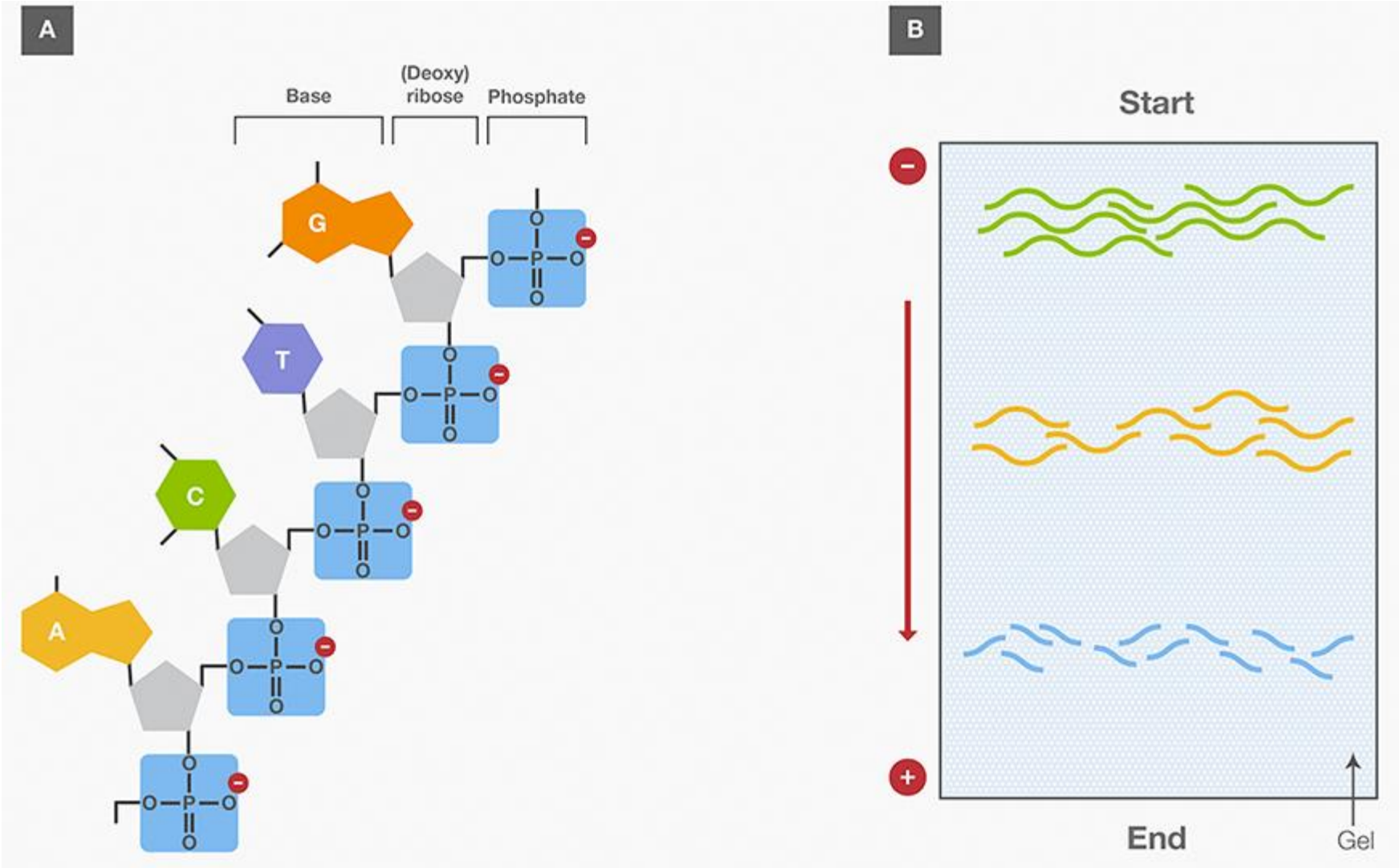
- The separation of macromolecules in an electric field is called *electrophoresis*
- Used to identify, quantify, and purify nucleic acids
- Nucleic acids separated in minutes to hours
- Electric field is applied to charged molecules causing them to migrate through gel matrix.
- Based on size, charge, and structure

Charge, size and shape

- Nucleic acids are negatively charged because of the phosphate groups of the ribose-phosphate backbones
- DNA or RNA molecules carry a constant charge-to-mass ratio.
- Mobility in gel is determined mainly on the basis of size
- Nucleic acids migrate from the negative electrode (cathode) toward the positive electrode (anode),
- Shorter fragments move more rapidly than longer ones
- For approximate sizing, samples are compared to molecular weight standards also called ladders/marker
- Ladders contain molecules of known sizes
- Highly compact supercoiled molecules migrate the fastest, followed by flexible linear and open circular molecules

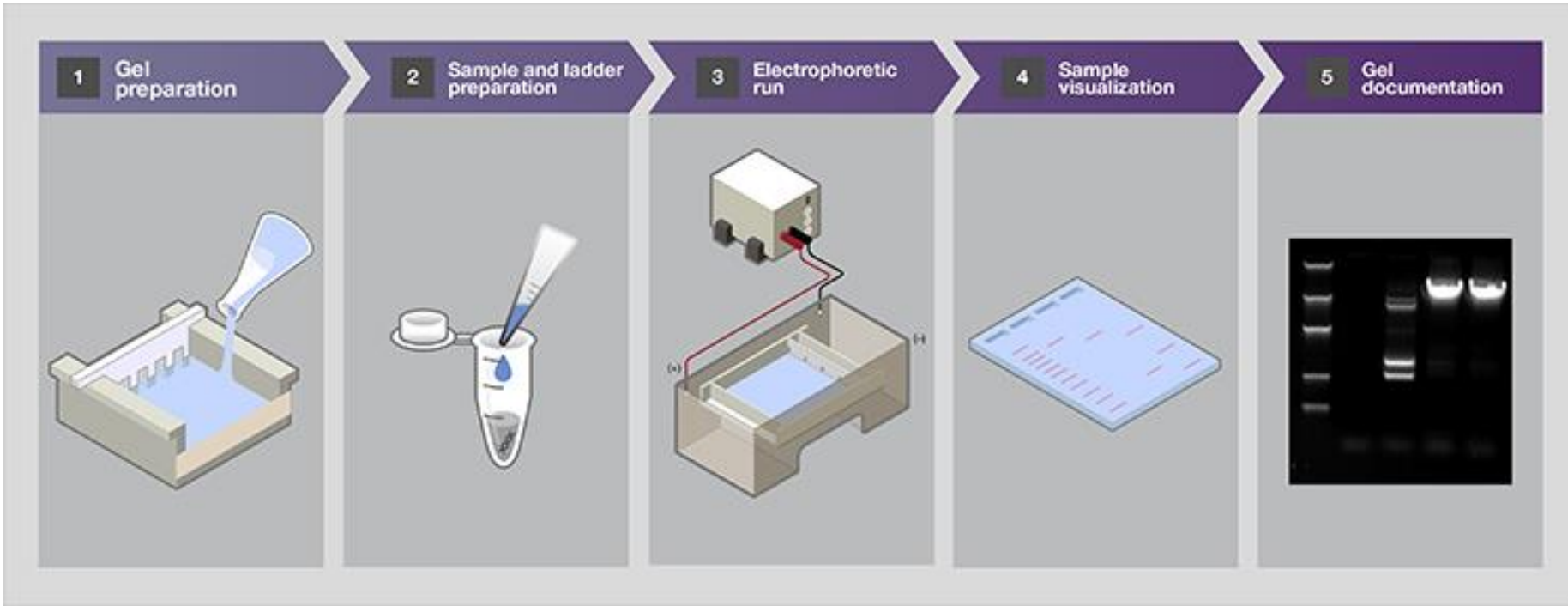


(A) Electrophoresis of nicked circular, linear, and supercoiled plasmid DNA. **(B)** Conformation of relaxed circular, linear, and supercoiled plasmid DNA



(A) Net negative charges carried by a nucleic acid chain. **(B)** Separation of nucleic acid fragments of varying lengths in gel electrophoresis.

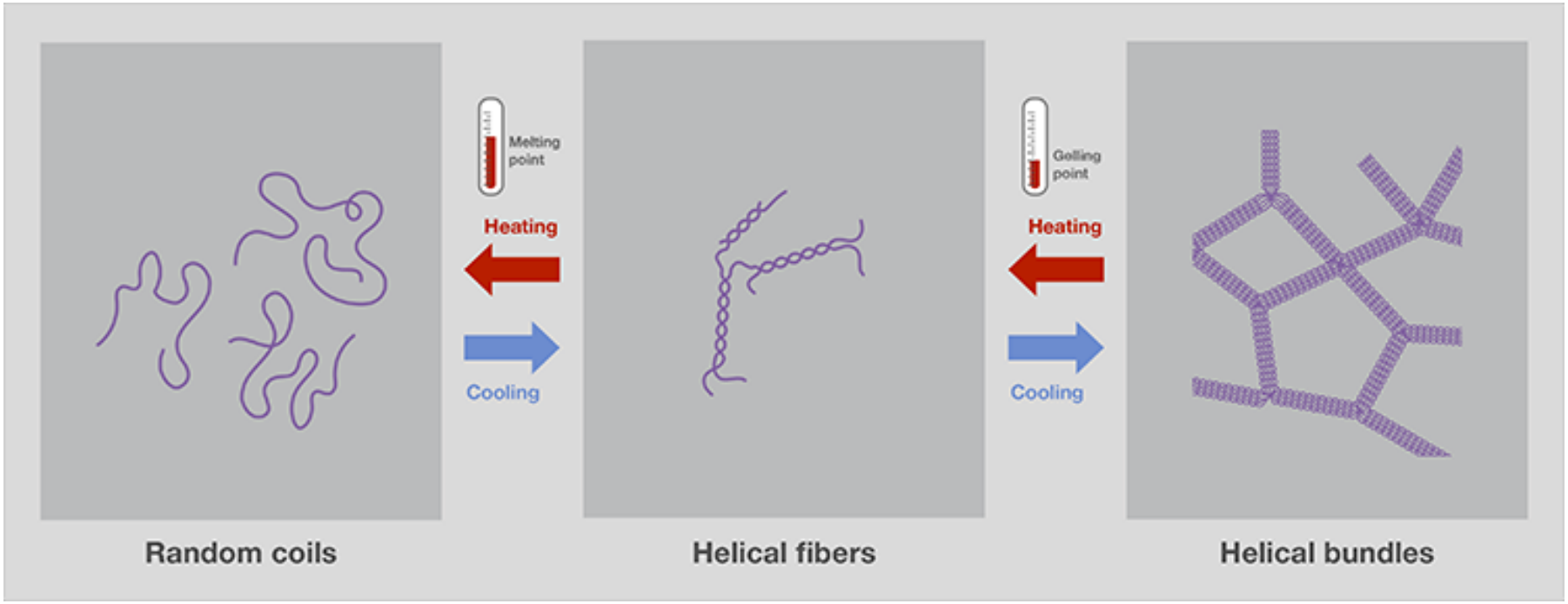
Agarose gel Electrophoresis Workflow



Agarose gel preparation

- Agarose is commonly available as powders
- Agarose is a purified form of agar, an unbranched (linear) polymer
- A gel matrix is formed, pore sizes ranging from 50 to 200 nm in diameter when heated and cooled
- Different agarose gel percentages used for the separation of DNA fragments of different lengths
- Low-percentage gels can be fragile and difficult to handle
- Usually 0.8% gel for genomic DNA visualization and 2% gel for PCR products.

- At a temperature above 90°C, agarose melts and becomes random coils.
- Upon cooling, two agarose chains form helical fibers linked by hydrogen bonds.
- Cooling below 40°C results in networks of helical bundles held together by hydrogen bonds, forming a gel with three-dimensional meshes



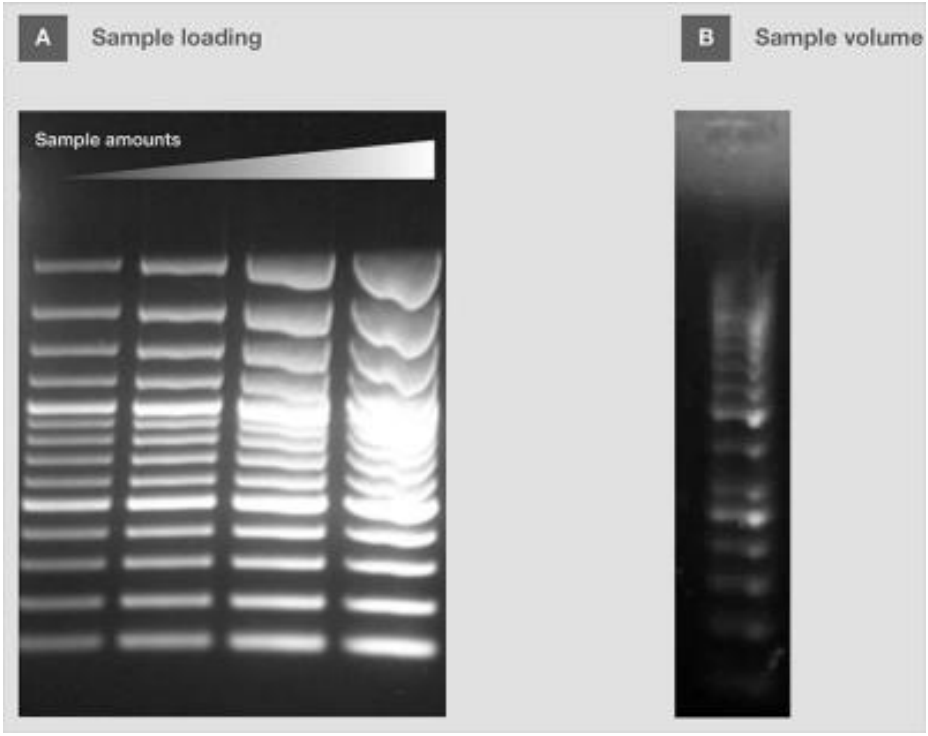
Agarose structure in solution, changed by heating and cooling

Buffer choice in gel preparation

- Ionic solution with electrical conductivity and buffering capacity are used to enable nucleic acid mobility during electrophoresis.
- Same buffer type is used for both the gel and the running buffer during an electrophoretic run
- To maintain the same pH and ionic strength
- Tris-acetate with EDTA (TAE) and Tris-borate with EDTA (TBE)
- Both have pH close to neutral to favor negative charges on the nucleic acids
- TAE: better for shorter runs- lower buffering capacity
- TBE: suitable for longer runs, separation of shorter fragments

Sample and standard preparation

- Overloading a sample can result in smearing of bands and masking those nearby resulting in poor resolution
- Using a smaller volume may result in band distortion, due to poor distribution in the well



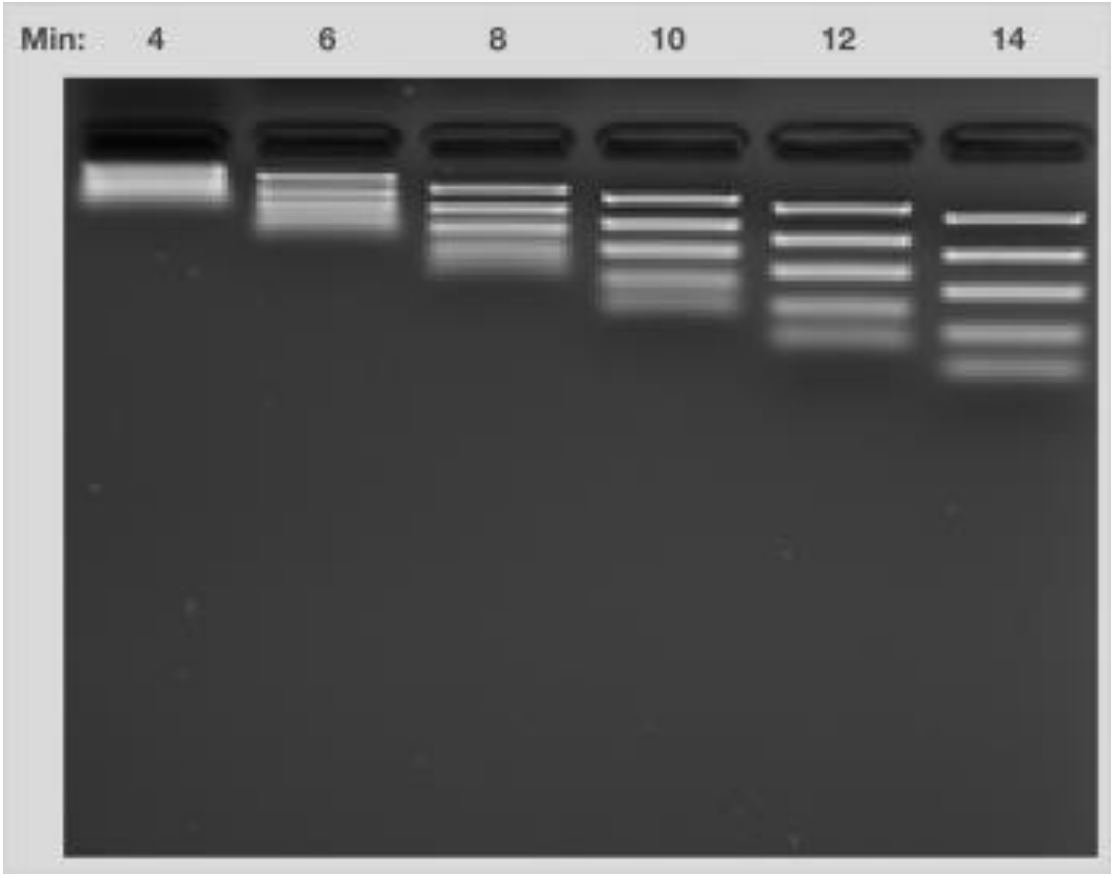
(A) The amount loaded affects band resolution. **(B)** A low volume of loaded sample may result in distorted bands.

Loading dye

- Samples and ladders are prepared in a loading dye
- Components of loading buffers:
- A **density ingredient**, such as glycerol or sucrose, increases viscosity of the samples
- **Salts**, such as Tris-HCl, create environments with favorable ionic strength and pH for the samples
- A **metal chelator**, such as EDTA, prevents nucleases present in the sample from degrading nucleic acids
- **Dyes** provides color for easy monitoring of sample loading, progress of the electrophoretic run
- Bromophenol blue (short products) or xyalene cyanol (large products)

Electrophoresis Run

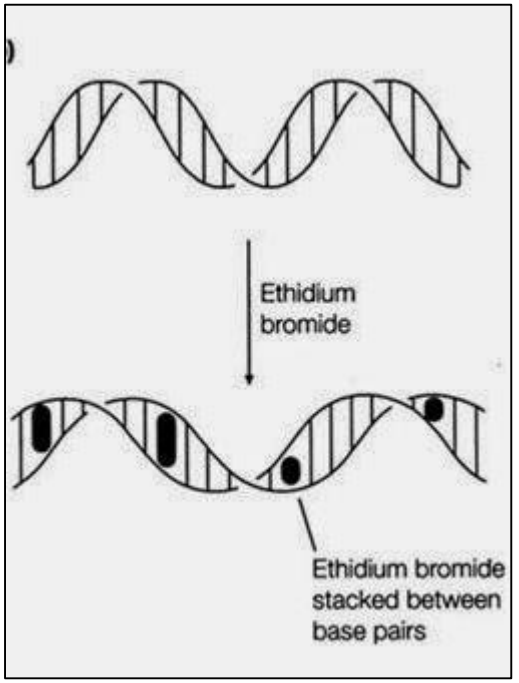
- Gel must be completely solidified
- Transfer the gel into tank
- Add buffer
- Remove comb
- Remove bubles from wells
- Gel should be completely submerged in the buffer
- Apply constant voltage
- Run time depends upon the length of the gel, the voltage used, and the sizes of the molecules in the sample



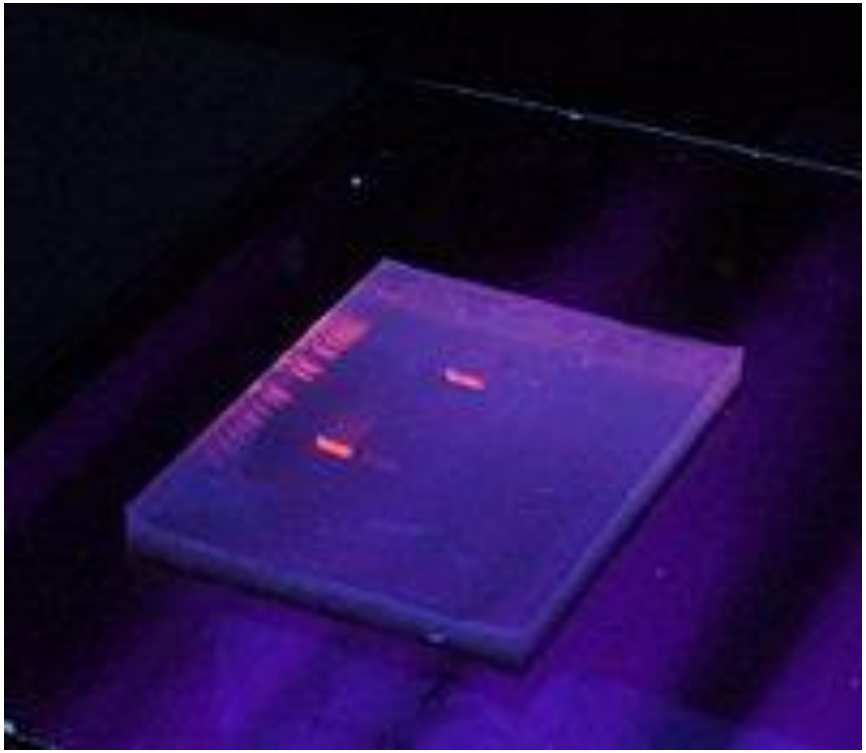
Effects of run time on sample separation.

Sample Visualizing

- To visualise samples, fluorscent dyes are usually used to detect nucleic acid.
- Added while preparing gel or the gels can be stained after the run is complete.
- Ethidium bromide is usually used for DNA, 0.2-0.5µg/ml
- It intercalates between the nitrogenous bases of **DNA** and fluoresces under UV light
- DNA can be observed under UV light.
- Gel red, safer alternative.



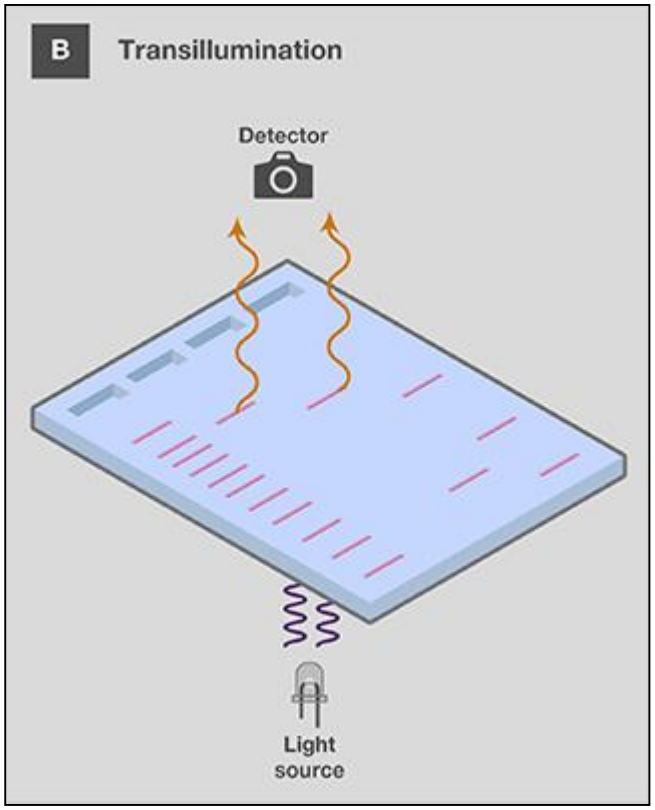
Ethidium bromide intercalated with DNA



DNA bands under UV light

Gel Documentation

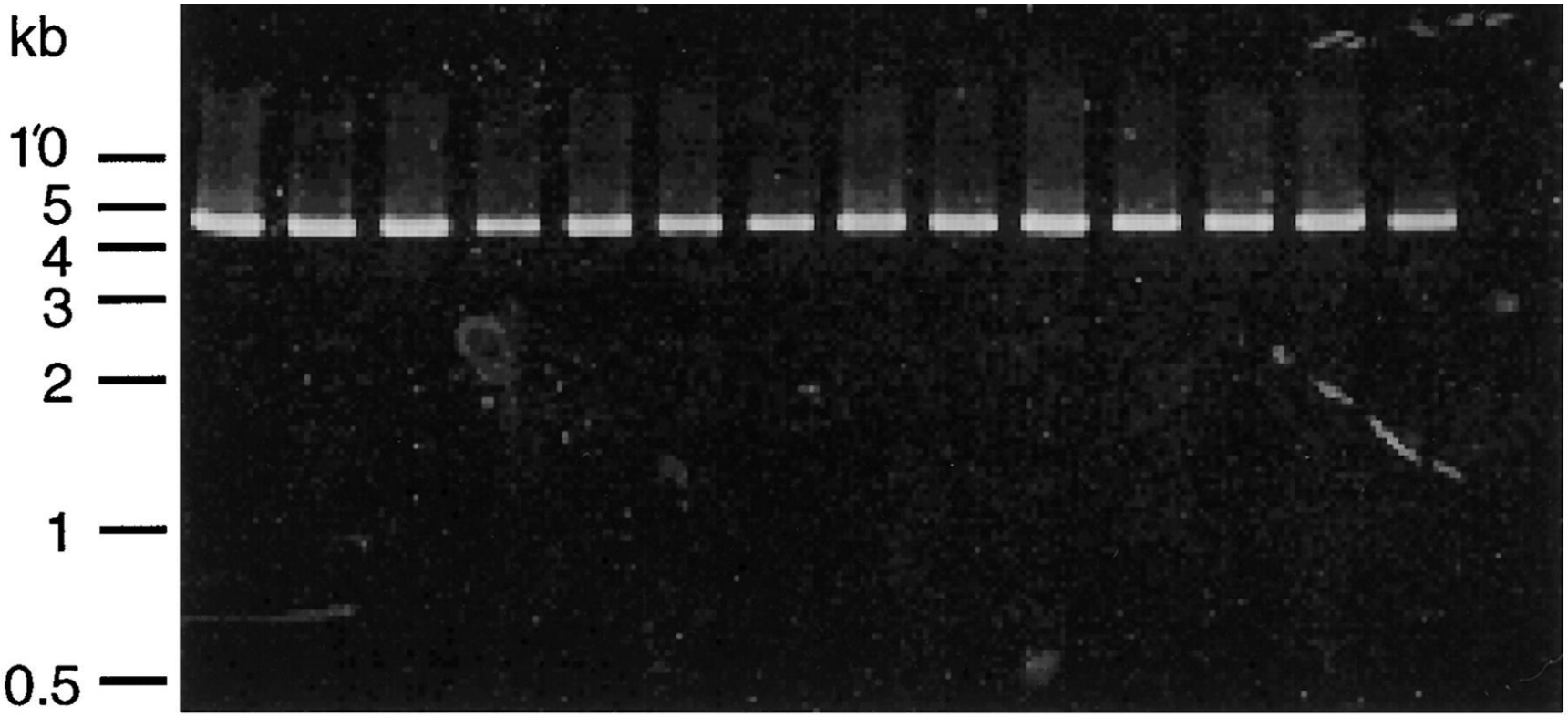
- After visualization, nucleic acid gels are typically documented for record and analysis
- Gels stained with fluorscent dyes are documented using gel document system containg iluminators



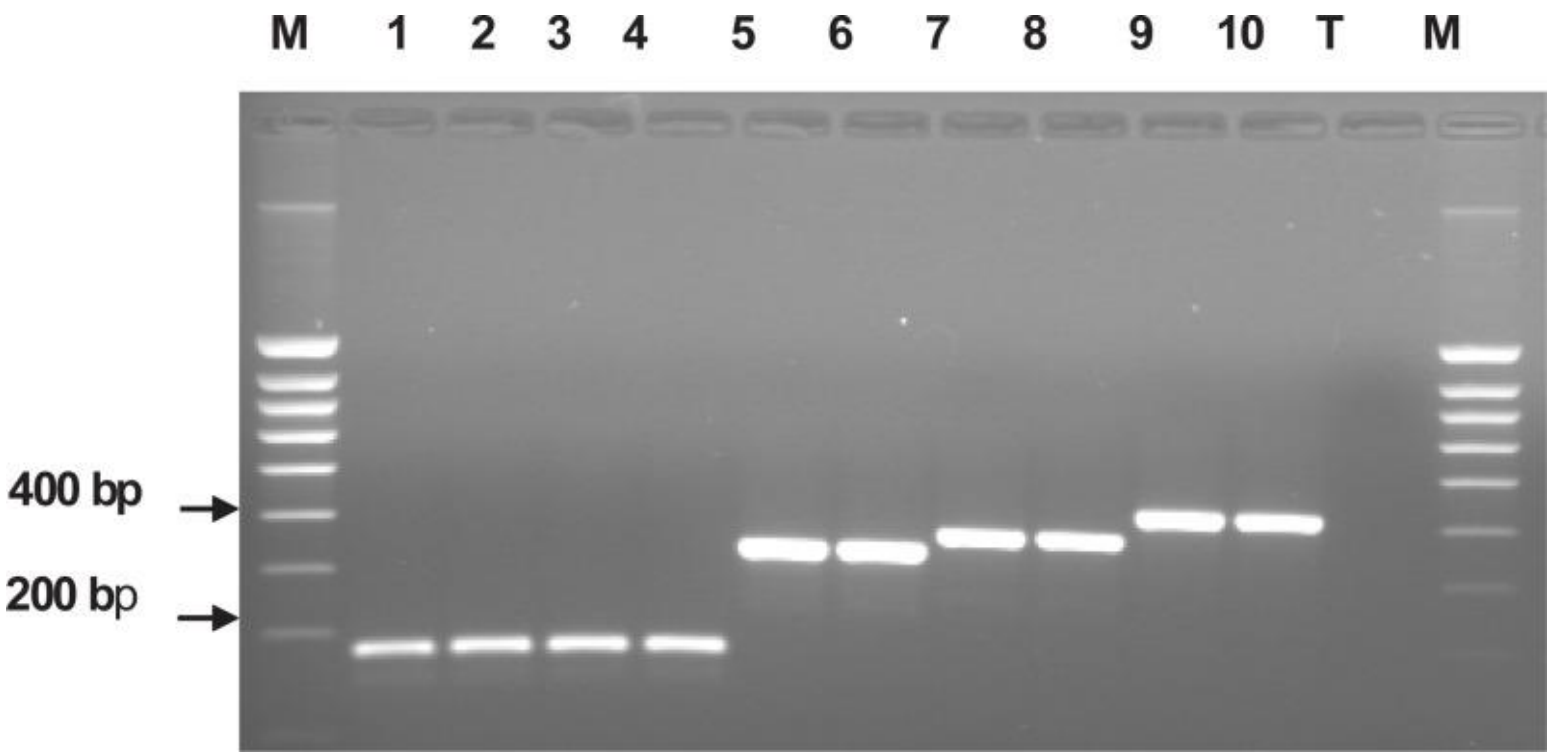
Gel documentation system

Agarose Gel electrophoresis

- https://www.youtube.com/watch?v=TIZRGt3YAug

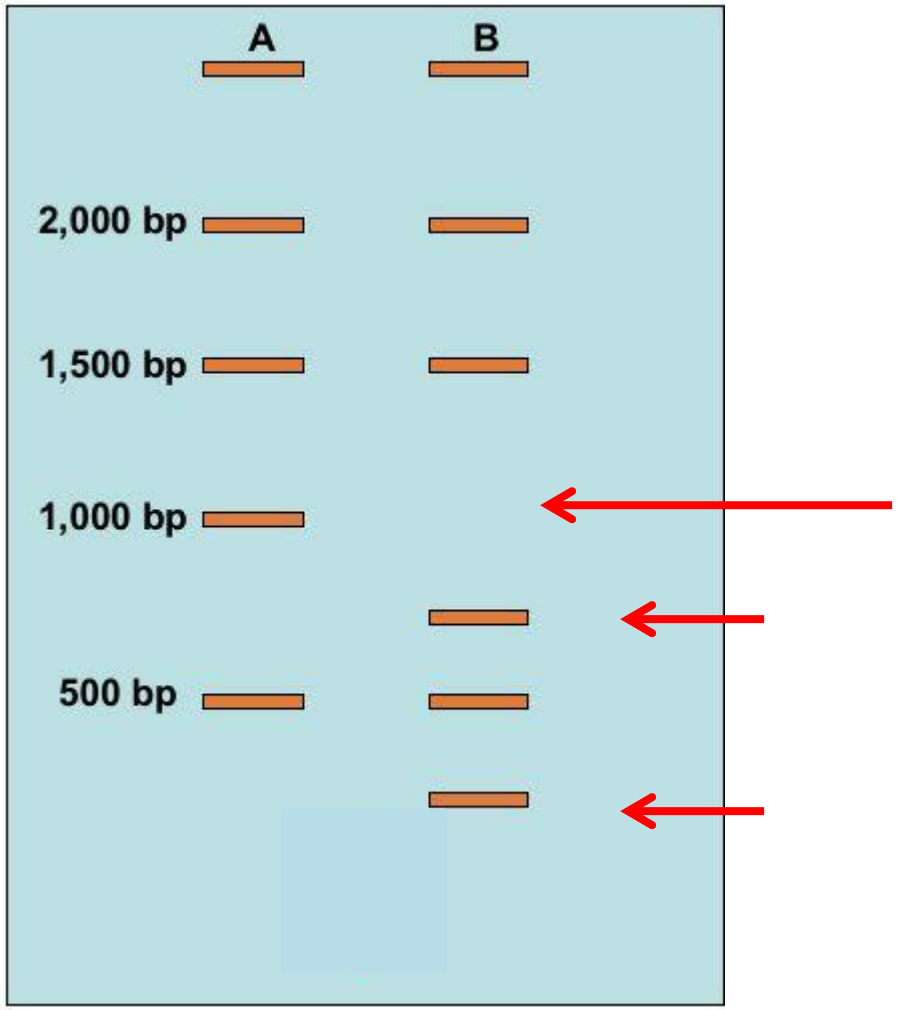


Isolated DNA bands



PCR products with different sizes

Restriction digestion



Thank you!