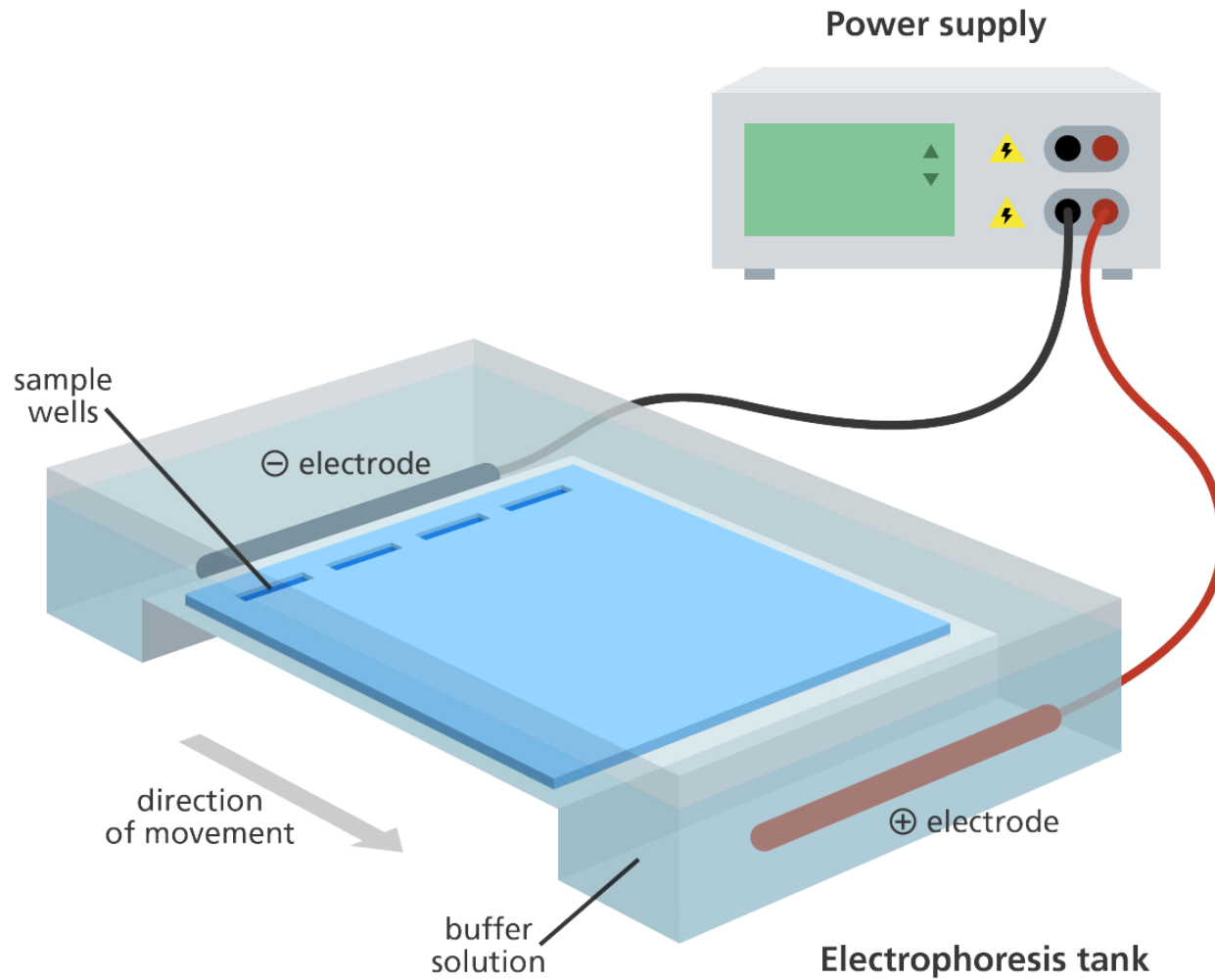


***in vitro* methods in studies of
nucleic acids**

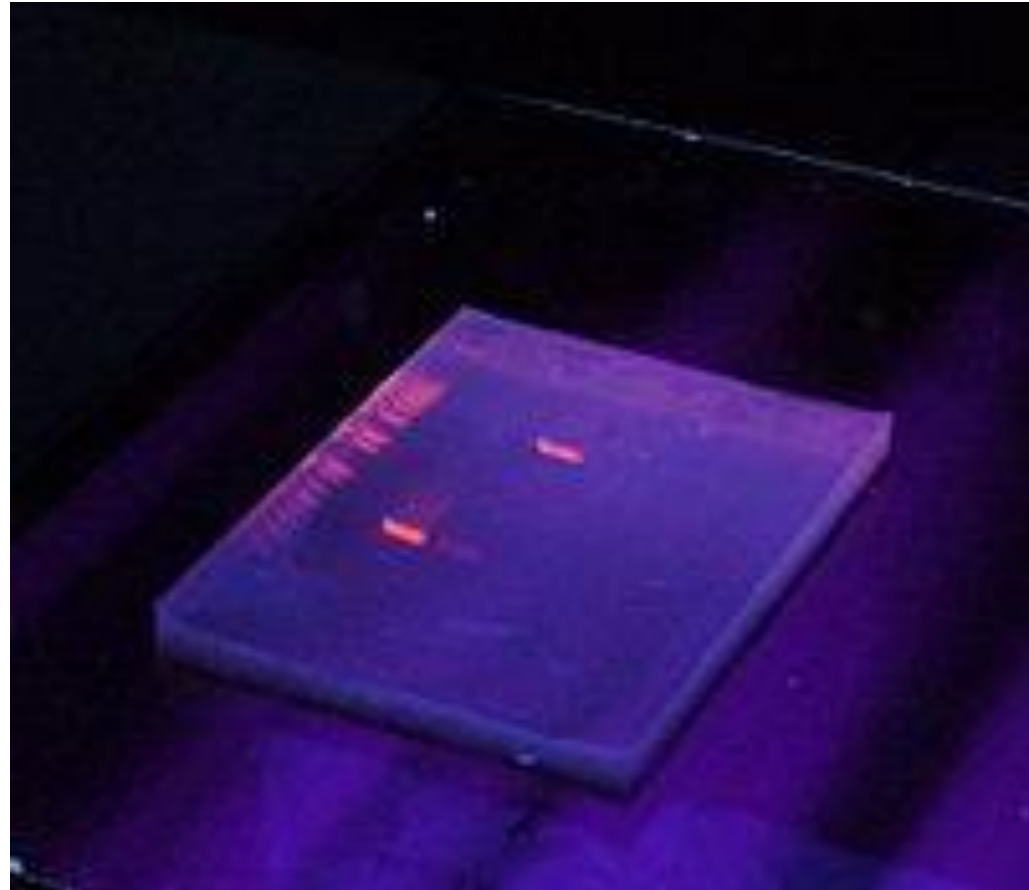
Separation of DNA

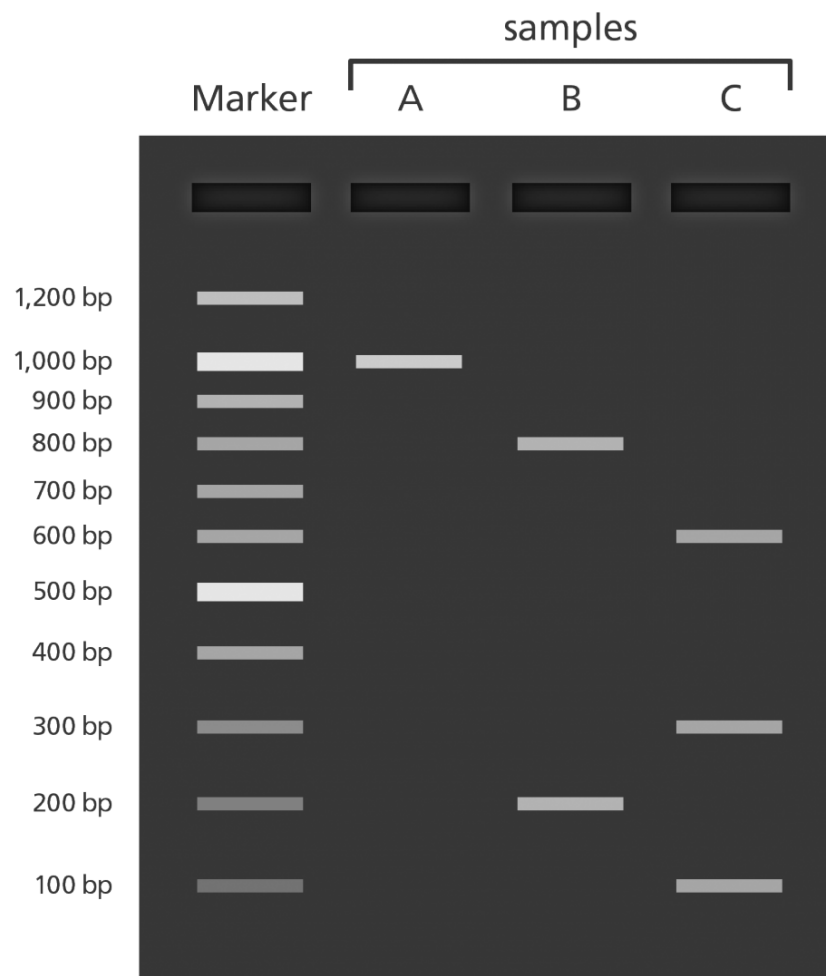
- DNA molecules could be separated by size using gel electrophoresis
- DNA molecules are negatively charged thus if electric field is applied to DNA solution the molecules will migrate to the positive electrode. This movement of molecules in electric field is called *electrophoresis*.

- The speed of the movement depends on the ratio of charge of the molecule to its molecular mass.
- DNA is a periodic molecule thus the charge to molecular mass ratio is approximately the same for all DNA molecules
- Thus, if there is no resistance on the way all DNA molecules would migrate with the same speed
- In order to separate DNA molecules by size the electrophoresis is performed in a gel a special porous medium. The size of the pores is comparable to the separating DNA molecules' size.
- Larger DNA molecules will encounter stronger resistance and migrate slower than smaller DNA molecules.



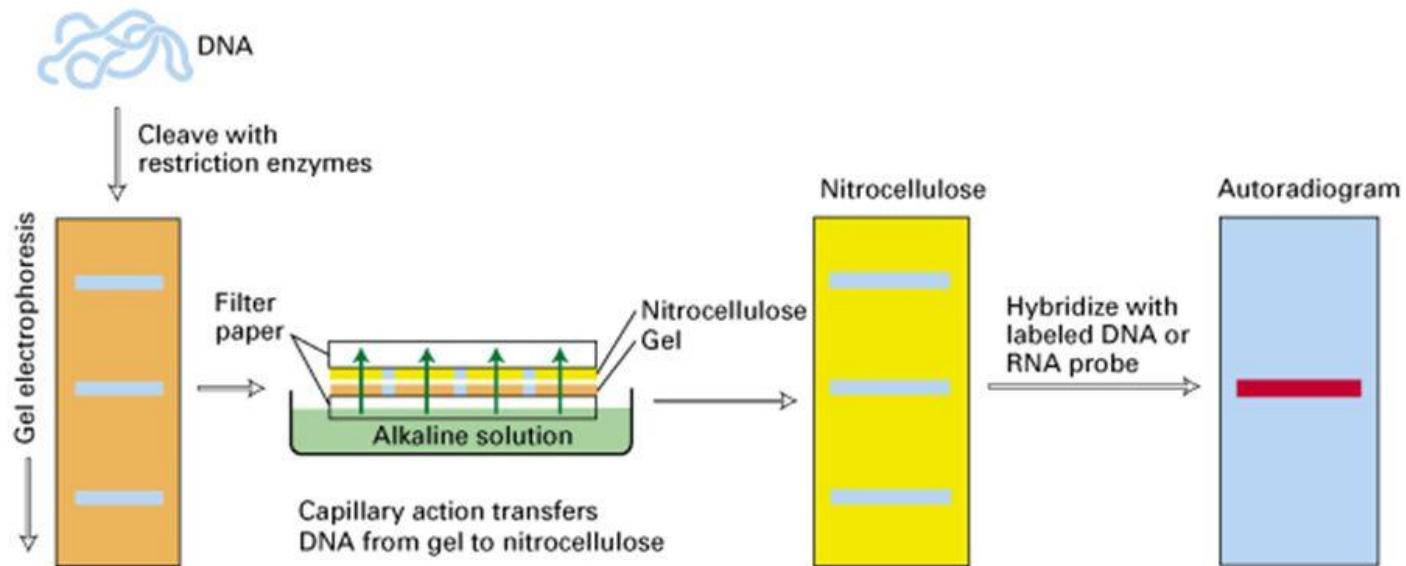
- DNA is visualized with special dyes that bind to it. Most commonly used is Ethidium Bromide – a fluorescent dye.
- DNA fragment size is determined by comparing the position of the fragment on the gel to the molecular weight marker.





- Staining with ethidium bromide or other fluorescent dyes allows to detect all DNA in the gel
- In some experiments DNA molecules with specific sequence need to be detected
- Commonly used method – Southern blotting and hybridization with labeled sequence-specific probe

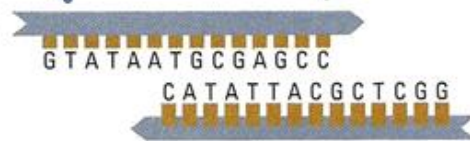
Characterization: Southern blot hybridization



- transfer of DNA from a gel to a membrane (e.g., nitrocellulose, nylon)
- developed by Edwin Southern

(A) Fragments of denatured and labeled probe DNA

The denatured probe usually contains *both* complementary strands.



(B) Fragments of denatured genomic DNA immobilized on filter

Renaturation

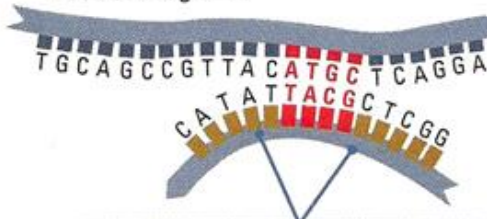
Some fragments in the genomic DNA may contain a sequence similar to that in the probe DNA.

Random collisions bring small regions of complementary sequences together to start the renaturation.

(C)

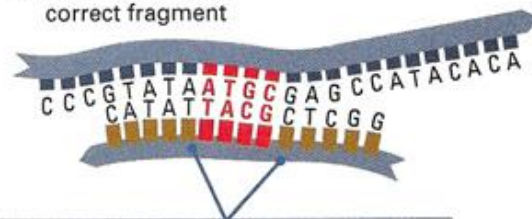
Heat-sealed bag

(D) Initial pairing with incorrect fragment



Base pairing cannot go farther because flanking sequences are not complementary; probe falls away.

(E) Initial pairing with correct fragment

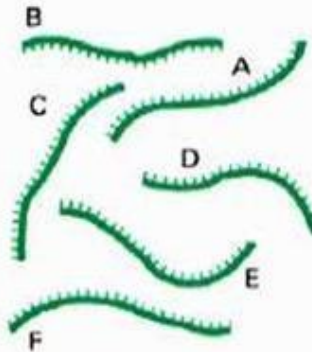


Base pairing proceeds in a zipper-like fashion because flanking sequences are complementary; probe sticks.

Single-stranded
DNA probes
for gene A

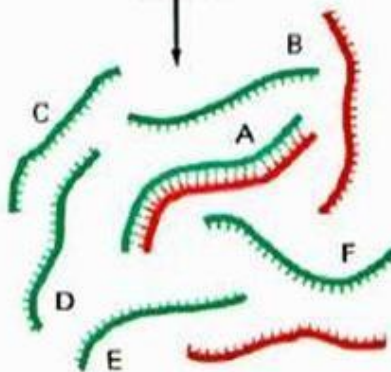


+



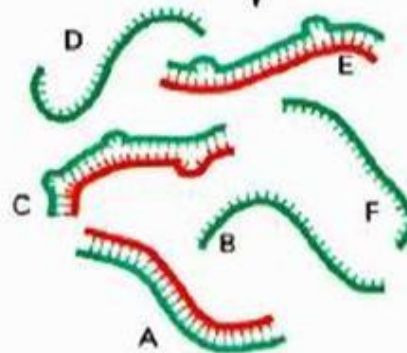
Mixture of
single-stranded
DNA molecules

Hybridization in
50% formamide
at 42°C



Only A forms stable
double helix

Hybridization in
50% formamide
at 35°C



A, C, and E all form
stable double helices

42 C
is more stringent
condition
than 35 C



**(hybridization
is more specific)**

