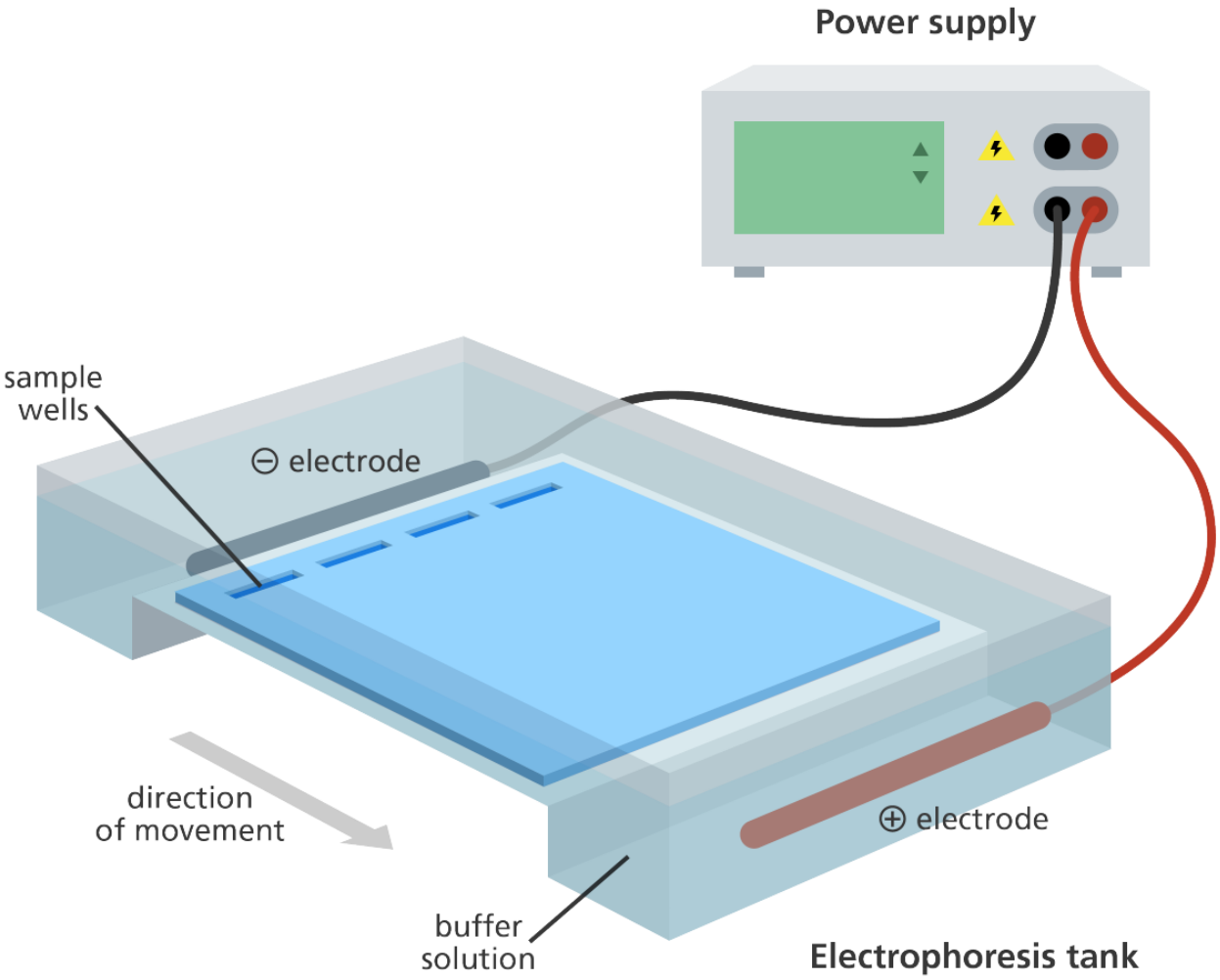
in vitro methods in studies of nucleic acids

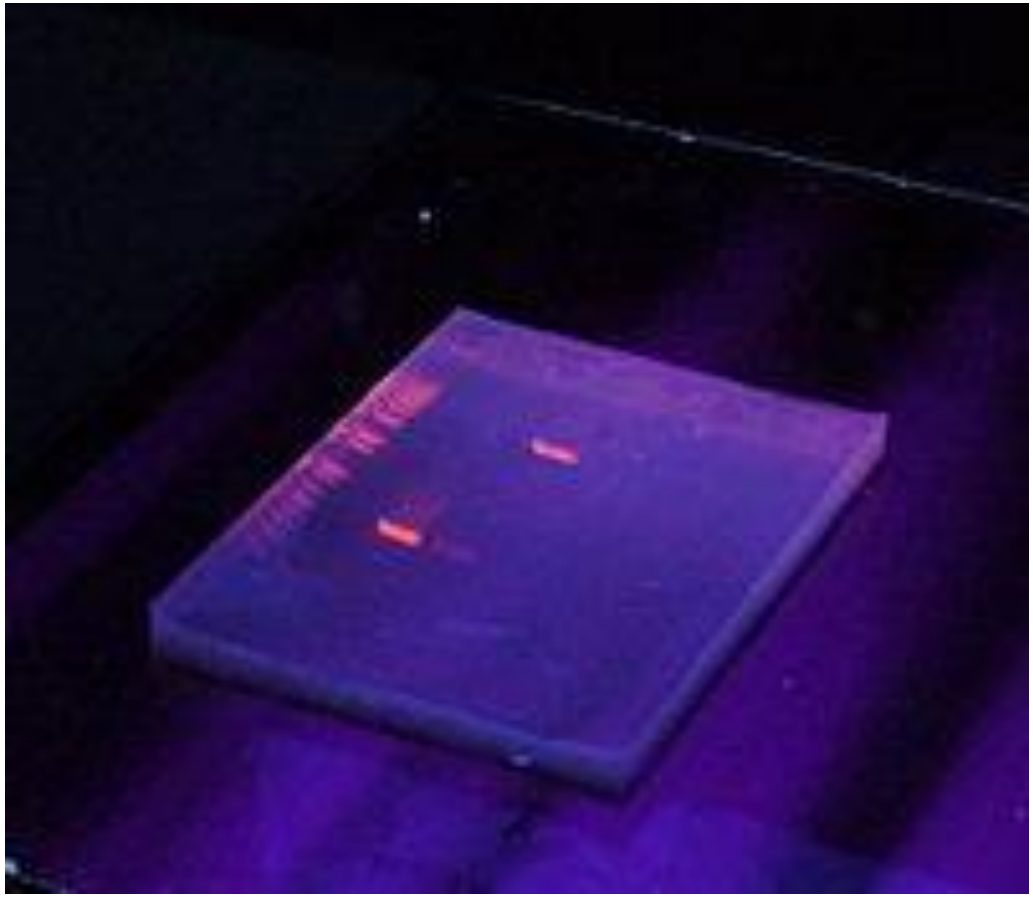
Separation of DNA

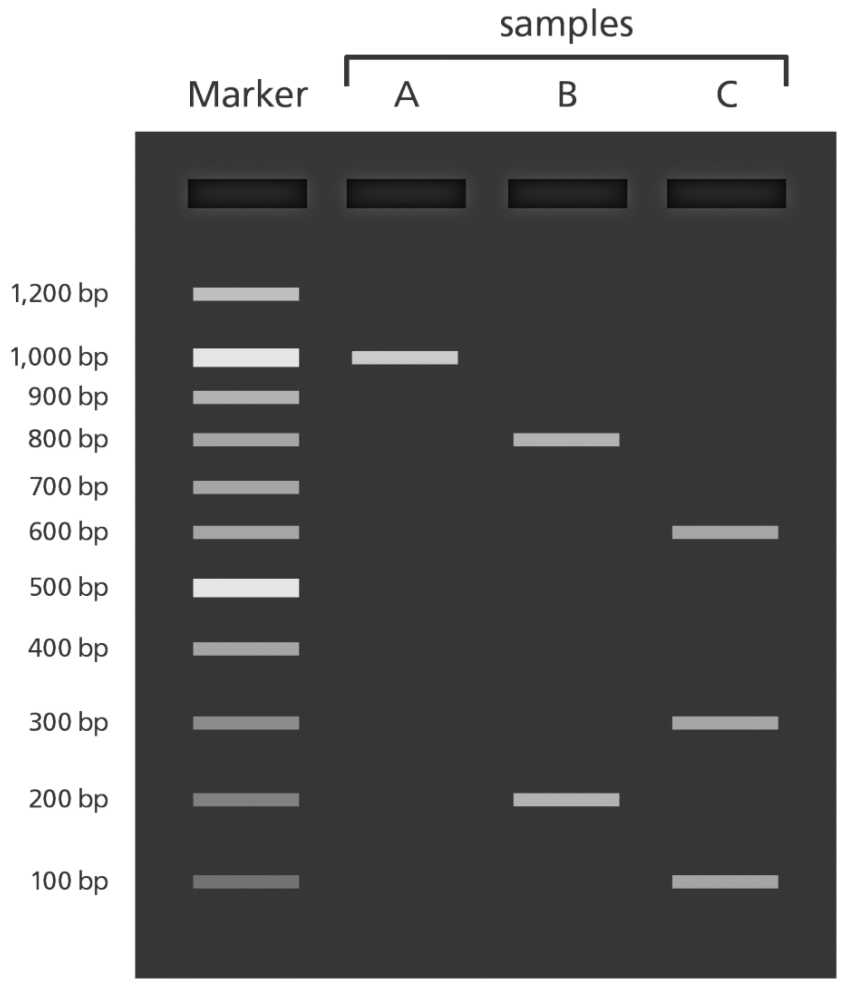
- DNA molecules could be separated by size using gel electrophoresis
- DNA molecules are negatively charged thus if electric field is applied to DNA solution the molecules will migrate to the positive electrode. This movement of molecules in electric field is called electrophoresis.

- 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.


Power supply
sample wells
⊖ electrode
direction of movement
⊕ electrode
buffer solution
Electrophoresis tank

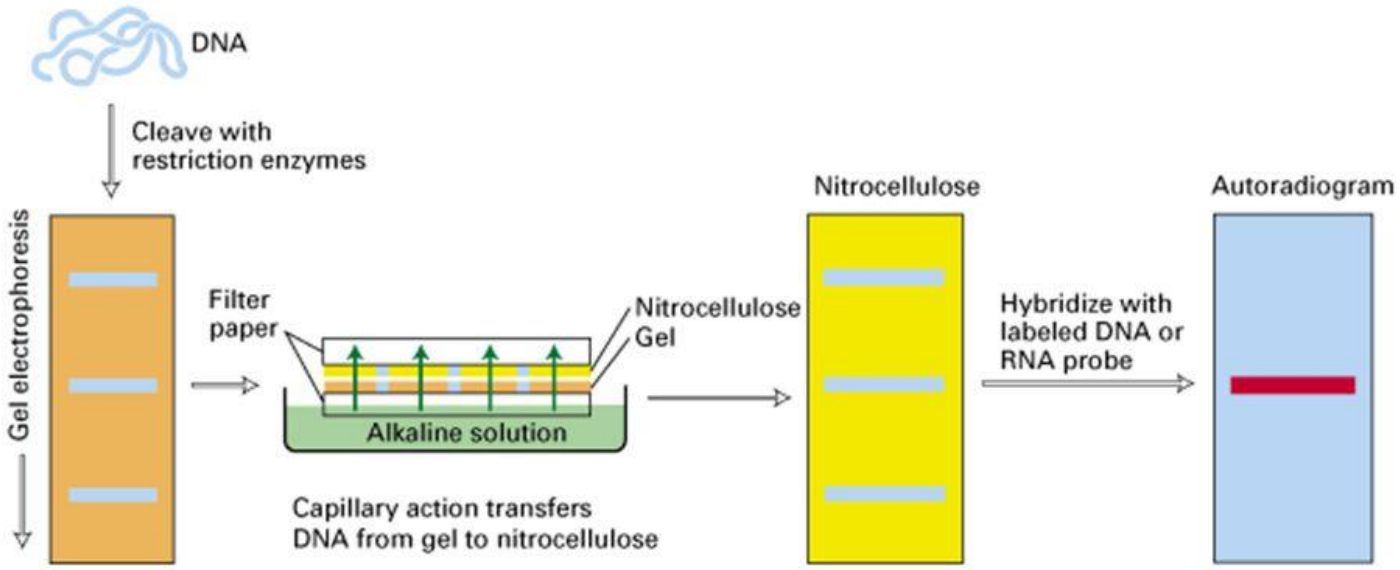
- DNA is visualized with special dyes that bind to it. Most commonly used is Ethidium Bromide – a fluorescent dye.
- DNA fragment size is determined by comparing the position of the fragment on the gel to the molecular weight marker.



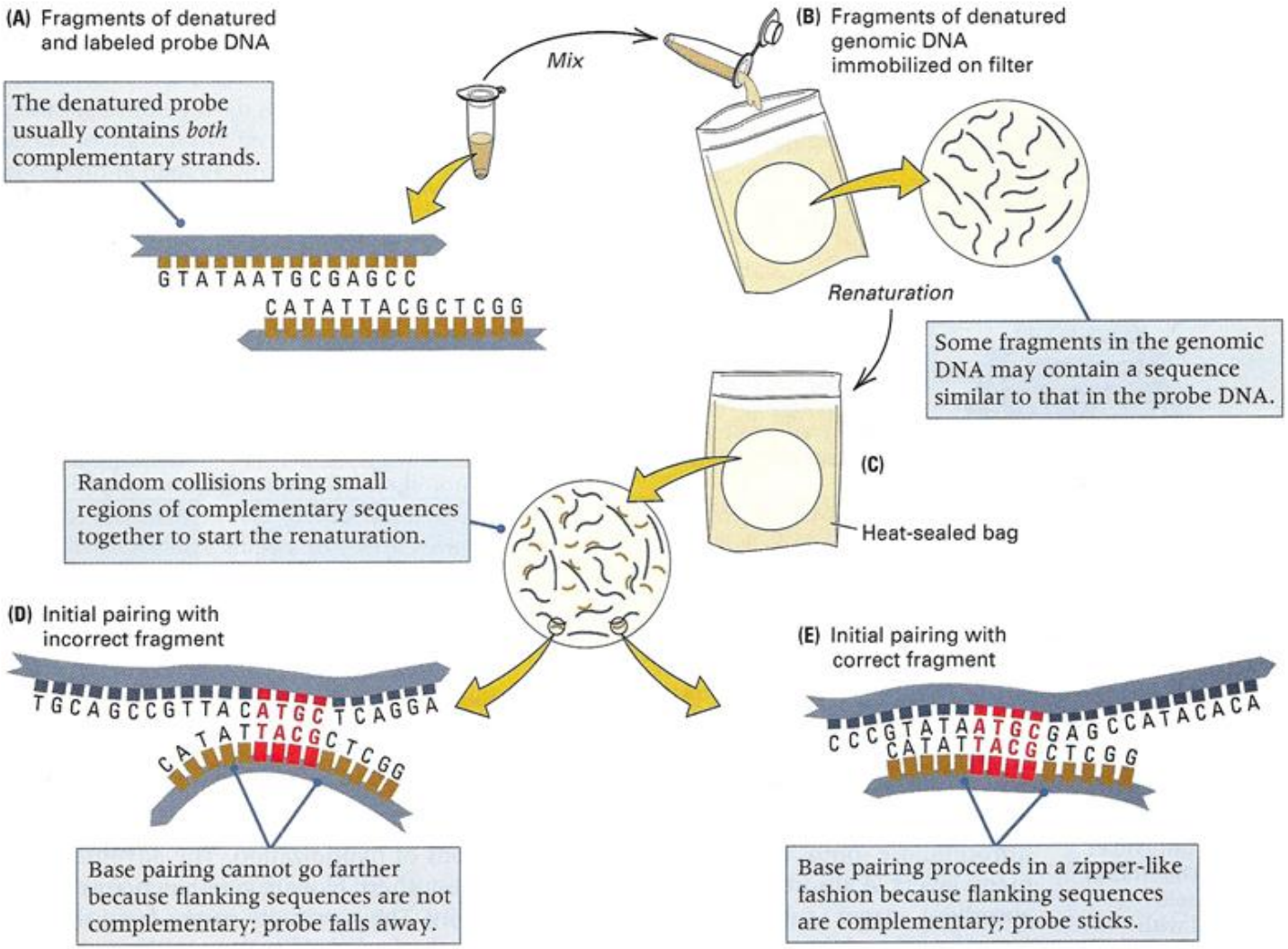

samples
Marker
A
B
C
1,200 bp
1,000 bp
900 bp
800 bp
700 bp
600 bp
500 bp
400 bp
300 bp
200 bp
100 bp

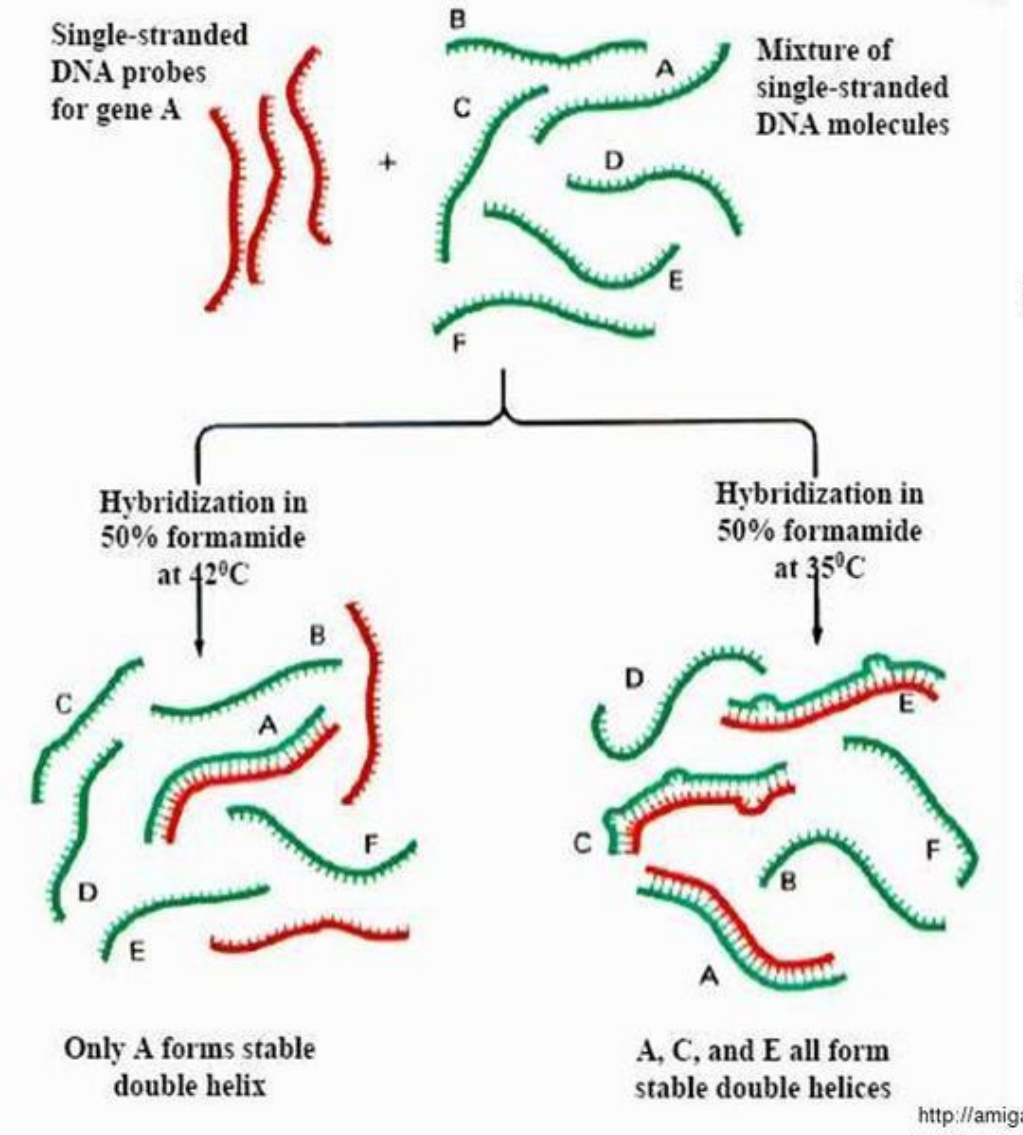
- Staining with ethidium bromide or other fluorescent dyes allows to detect all DNA in the gel
- In some experiments DNA molecules with specific sequence need to be detected
- Commonly used method – Southern blotting and hybridization with labeled sequence-specific probe

Characterization: Southern blot hybridization



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

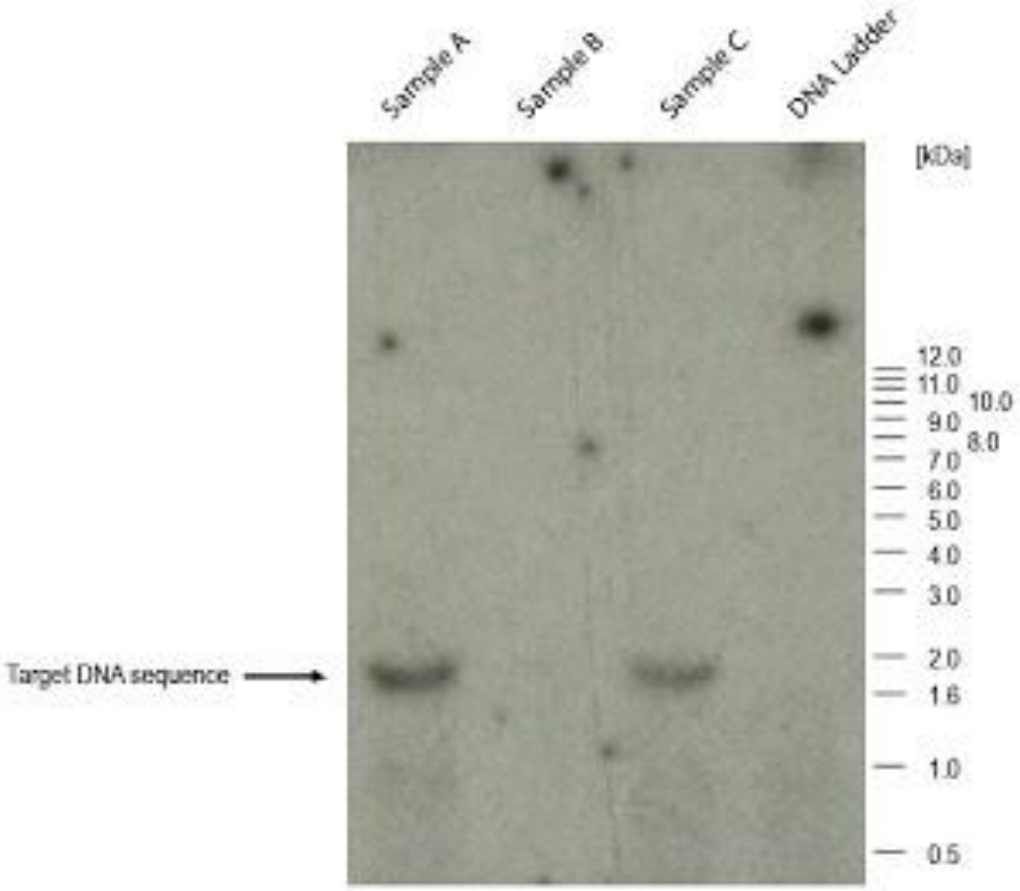

(A) Fragments of denatured and labeled probe DNA
The denatured probe usually contains *both* complementary strands.
GTATAATGCGAGCC
CATATTACGCTCGG
Mix
(B) Fragments of denatured genomic DNA immobilized on filter
Renaturation
Some fragments in the genomic DNA may contain a sequence similar to that in the probe DNA.
(C)
Heat-sealed bag
Random collisions bring small regions of complementary sequences together to start the renaturation.
(D) Initial pairing with incorrect fragment
TGCAGCCGTTACATGCTCAGGA
CATATTACGCTCGG
Base pairing cannot go farther because flanking sequences are not complementary; probe falls away.
(E) Initial pairing with correct fragment
CCCGTATAATGCGAGCCATACACA
CATATTACGCTCGG
Base pairing proceeds in a zipper-like fashion because flanking sequences are complementary; probe sticks.



42 C
is more stringent
condition
that 35 C

(hybridization
is more specific)

http://amiga1.med.miami.edu/Medical/PDF-Files/Lecture10.pdf


Sample A
Sample B
Sample C
DNA Ladder
[KDa]
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.6
1.0
0.5
Target DNA sequence